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ACTA PHYSIOLOGICA SCANDINAVICA
VOL. 47 SUPPLEMENTUM 163

SITE OF PRODUCTION OF OESTROGEN IN RAT OVARY AS STUDIED IN MICRO-TRANSPLANTS

BY
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ACTA PHYSIOLOGICA SCANDINAVICA
VOL. 47 SUPPLEMENTUM 163
FROM THE DEPARTMENT OF HISTOLOGY,
UNIVERSITY OF LUND, LUND, SWEDEN

Site of Production of Oestrogen in Rat Ovary as Studied in Micro-Transplants

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LUND 1959

Translated by Mr. L. James Brown

Printed in Sweden

BERLINGSKA BOKTRYCKERIET
LUND 1959

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Introduction

Opinions still differ on the site of production of oestrogen in the ovary — a basic problem of the ovarian physiology. This lack of unanimity is largely ascribable to the fact that as yet only indirect and often contradictory evidences have been produced in support of the various theories available.

The present paper is concerned with an investigation of this problem. For this purpose an experimental method was designed to yield more direct evidence by placing rat ovarian microtransplants made up of only one or more than one endocrine cell system contiguous to a vaginal graft, serving as a sensitive indicator of steroids with oestrogenic effects.

Chapter I

Material and Methods

ANIMALS

In the experiments to be described it was necessary to use animals possessing a target organ reacting to oestrogen in a specific and readily recordable way. The most suitable organ satisfying these requirements is the vagina of certain rodents. On stimulation with oestrogen the vagina in these animals reacts with the formation of cornified stratified epithelium. Of this group of animals the rat is the one that has been most extensively studied as an experimental animal, which is of importance for the interpretation of the experimental results. In a simultaneous comparative investigation of the function of the interstitial cells of the ovary in different species, the rat was also found to be the most suitable animal from a technical point of view.

In the present investigation normally cycling female albino rats, weighing 150—230 grams, were used.

Most of the animals were Sprague Dawley rats, but the experimental series also included rats of other stocks. No difference was found between the results obtained in the Sprague Dawley rats and the remainder. The animals were fed a well balanced commercial brand of laboratory food (AB Anticimex, Stockholm) and were allowed to drink water *ad libitum*. It was checked that the animals were normally cycling by studying at least five cycles by daily vaginal smears taken by a small cotton pledget moistened with physiological saline.

PRINCIPLE OF THE EXPERIMENTS

In the elaboration of the method for direct registration of ovarian oestrogen secretion, it was thought that the method should preferably fill the following requirements.

1. It should permit examination of hormone production by an ovarian graft consisting of one or more well defined cell systems and transplanted to a spayed rat.

2. The method should include an indicator system with high specificity and sensitivity to oestrogens. The indicator should record only the oestrogen primarily formed in the ovarian graft and should therefore respond directly to the local action of the oestrogen.

3. The production of oestrogens by the transplants should not be of such an order as to give rise to any systemic effect and thereby disturb separate investigation of the response of the epithelium of a vaginal transplant inserted at some site other than that of the ovarian graft if stimulated by some other organ *e.g.* adrenal cortex or metabolites from the primary steroids produced by the transplant. It cannot be excluded that the ovary might be able to produce steroids not themselves oestrogenic but capable of being metabolised to substances with an oestrogenic effect. Otherwise it would not be possible to know whether the response of the indicator cells is produced directly by the local action of oestrogen from the ovarian graft.

4. The experimental period should be as short as possible, long-term experiments involving the risk of morphological and functional changes of the cells transplanted (*cf.* Chapter VII). Such alterations would probably make it difficult to identify the cells in the transplant and possibly also result in a changed steroid formation. In addition, it is not known whether prolonged excessive stimulation by the pituitary gonadotrophic hormones in spayed animals might not lead to alterations in the steroid formation.

5. It should preferably not be necessary to use exogenous gonadotrophic stimulation, the choice of gonadotrophic hormone preparation and dosage being difficult. Since the effect of the various pure gonadotrophic hormones on the steroid formation by the ovaries is by no means properly understood, gonadotrophic stimulation solely by the animal's own hypophysis was considered desirable.

These requirements dictated the design of the experimental method. A dissection technique was devised which permits pre-

paration of small aggregates of cells containing only one of the endocrine systems of the ovary. Such systems were transplanted alone or in combination. The production of hormones by such small grafts hardly ever produced any systemic effect on the uterus and vagina *in situ*. A suitable indicator system was secured by transplanting a piece of the vaginal mucosa of the experimental animal contiguous to the ovarian graft.

TRANSPLANTATION METHODS

The pieces of tissue were always transplanted to the anterior chamber of the eye. The advantage of this site of transplantation is that even very small grafts can be readily identified there and that they are always well outlined against the iris and can be excised as a whole without any undue amount of adherent tissue from the site of the transplant. This facilitates subsequent serial section of the specimen.

In addition, preliminary experiments had shown that non-sterile pieces of vaginal tissue from the adult animals often gave rise to local inflammation when they were transplanted to the muscle, for example, but seldom when grafted to the anterior chamber of the eye.

It was originally assumed that in short-term experiments it would be difficult to record the effect of oestrogen on vaginal epithelium already under the influence of the ovarian hormones at the time of transplantation (*cf.* Chapter II). In the first experiments ovarian tissue from the adult rat was therefore transplanted together with a sterile vaginal autotransplant to immature recipient animals with closed vagina and spayed at least 10 days previously. Transplantation of the vaginal pieces to the musculature of the back was never followed by any signs of local inflammation. But the homologous ovarian grafts showed a very low survival in experiments of 4—12 days' duration: only some of the large grafts consisting of one third to one half of the ovary survived this period, but they caused a more or less severe round cell infiltration. In one of the series autologous ovarian transplants were used together with homologous

vaginal mucosa from spayed immature rats. During experimental periods of 4—12 days these vaginal grafts survived more frequently than the homologous ovarian transplants, but round cell infiltration was fairly common with a tendency to produce non-specific epithelial reactions as a consequence. Homologous transplantation was therefore discontinued and only autologous grafts were used in the present study.

Operative procedures. Both ovaries were removed under ether anaesthesia. After removal of bursa and fatty tissue, they were rinsed in Krebs-Ringer-phosphate solution (see COHEN 1951) and transferred to a black porcelain crucible. Black had proved the best background colour for microdissection. Dissection was then done without delay under a Zeiss stereomicroscope (×12—80) with a heat filter (Schott BG 17) before the source of light. The ovaries were dissected with straight tweezers with very sharp pointed ends and a pair of scissors (Aesculap C 2247), the tips of which had been ground as fine as possible. With these scissors it was possible to dissect even very small aggregates of cells without mechanical distortion of the cells and consequent curtailment of their survival in the transplant. Preliminary experiments had shown such distortions to be unavoidable on dissection of the specimen with microneedles and to be very injurious to theca interna and interstitial gland cells, which seldom survived after such treatment. Not until suitable microscissors had become available could the cells be dissected without distortion. The precision of the scissors was such that a single lobe of interstitial gland could be excised.

Throughout the procedure, from the removal of the ovaries until the insertion of the graft into the anterior chamber of the eye, which seldom required more than 10 minutes, the ovaries and the transplants were kept in Krebs-Ringer-phosphate solution at room temperature.

The extreme susceptibility of the ovarian graft to mechanical traumata (see Chapter V) precluded the use of tweezers or the like for insertion of the graft into the eye. The pieces of tissue were therefore transferred to the eye by means of a pipette. The pipette consisted of a thin-walled glass tube, so small that it could be readily passed through a small corneal incision. In

order to prevent the iris from adhering to and being damaged by the pipette, the tip of the latter was cut obliquely and the edges were rounded off in a micro-flame. The pipette was connected with a plastic tube to an Agla microsyringe and the entire system was filled with Krebs-Ringer-phosphate solution. For controlling the delivery of the transplant it was found useful to fasten the syringe piston to that of the micrometer.

The eye to be operated upon was pushed out of the socket by digital pressure above and below the eye, and the anterior chamber was opened by a small incision near the upper corneoscleral junction, care being taken to avoid any damage to the iris with consequent haemorrhage making insertion of the graft more difficult. Despite extreme care, bleeding from the iris was sometimes unavoidable. But such haemorrhage was slight, the blood was usually soon absorbed, and had no demonstrable effect on the survival of the graft.

The micro-transplant was then sucked up into the pipette and introduced into the anterior chamber of the eye, after which it was gently fed to the anterior lower quadrant near the corneoscleral junction by gentle pressure against the cornea.

The vaginal graft was prepared in the following way. The vagina was carefully washed a few times with cotton pellets soaked in Krebs-Ringer-phosphate solution and dried gently with a cotton pellet. The vaginal opening was widened with a fine pincette. A pincette was then introduced into the vagina avoiding the perineal part, where the epithelium is constantly partly cornified, and the wall was gripped. A thin strip of epithelium with some of the underlying connective tissue was cut out. Two small pieces were then cut from the cervical end of the strip, one for transplantation to the left eye and one to the right. A third piece was cut off and examined histologically to check that the graft had been removed from a site remote enough from the vaginal opening. The vaginal graft was transferred from the Krebs-Ringer-phosphate solution by means of tweezers to the corneal incision, after which it was carefully introduced into the anterior chamber of the eye with the aid of a fine glass needle and then fed down by gentle pressure against the cornea until it was contiguous to the piece of ovarian tissue.

The intruments used were sterile, but no precautions were taken to secure strict asepsis, preliminary experiments having shown such precautions to be unnecessary.

SPECIAL METHODS FOR MICRO-DISSECTION OF OVARIES

The topography of the different structures was studied in serial sections of ovaries removed in the various stages of the oestrous cycle (the follicles being studied especially in pro-oestrus). On the basis of these studies the various structures were dissected, fixed and examined in serial sections. The results showed that it was possible to dissect cell aggregates made up of a single endocrine cell system or of a single cell system together with one or more desired structures. Such controls of the dissection technique were used throughout.

Follicle wall transplants

Serial sections of ovaries removed in the pro-oestrous phase showed that it was possible to select prominently bulgent follicles and excise pieces from their cupolas containing fairly abundant theca interna cells and granulosa cells. Such follicle wall pieces were often not contaminated with other structures except germinal epithelium, which could not be avoided and which, of course, applies to all transplants taken from the surface of the ovary. These grafts were dissected simply by excising that part of the top of the follicle where the wall was thinnest. For comparison, in some cases, where somewhat smaller follicles protruded so markedly from the surface of the ovary that their tops could be excised without any adherent tissue, they were transplanted instead of tops of the largest follicles.

Corpus luteum cell transplants

Aggregates of corpus luteum cells could be readily obtained from the interior of the corpus luteum. In other experiments tops of large corpora lutea were transplanted.

In most of the experiments the animals were in late oestrus, and the transplants were taken from the last corpus luteum

generation, in which the production of steroids might be thought to be most active. These corpora lutea were identified by type of vascularisation and presence of ovulation stigma and antrum (BASSET 1943). Whole corpora lutea were never transplanted, preliminary experiments having shown that they can never be dissected free from contaminating structures.

Granulosa cell transplants

Large follicles were punctured and granulosa cells were gently expressed. In this way large quantities of loose conglomerates of granulosa cells were obtained from the ovaries. As a rule, these transplants were taken from ovaries in the pro-oestrous phase.

If due care is exercised, the small conglomerates of cells can be pipetted off in coherent masses. They cannot, however, simply be pipetted into the anterior chamber of the eye because they will then seldom take on the iris. On the other hand, they will take and survive well, if they are deposited and retained on a small region of the iris. Such an accumulation of the cells was secured by giving the vaginal transplant the shape of a thin string and placing it like a bow on the iris, with the ends of the bow close to the corneo-scleral junction. The pipette used for this purpose was somewhat different from that described previously: it had a tapering, gently bent tip, which was cut obliquely and micro-flamed in the way described above. By introducing the tip into the bay formed by the vaginal graft and pipetting the cells slowly, good yields of surviving cells were obtained. A certain backflow and escape of cells during transplantation was unavoidable.

Interstitial cell transplants

Serial sections of normal ovaries showed that the central parts of the ovaries contain abundant gland lobes of interstitial cells, often well outlined by ovarian stroma. On the other hand, follicles are scanty. Control micro-dissection proved that gland lobes containing only interstitial cells could be obtained, but that the excised lobes were often contaminated with adherent tissue.

The ovary was divided through the hilus with a razor blade and the exposed surface was inspected for an interstitial gland lobe that appeared to lie isolated in the stroma without any observable follicles.

Such transplants were removed from ovaries in various stages of the oestrous cycle. Attempts to excise interstitial cells from the surface of the ovary resulted in a marked increase in the frequency of contaminated transplants.

Theca interna cell transplants

One of the most difficult problems was to devise a method for separating granulosa cells from theca interna cells without damaging the latter by the separation procedure, or at least not so severely that they did not survive on transplantation.

For these transplants the cupolas of the largest follicles in pro-oestrous ovaries were used. They were dissected in the way described above with the exception that the excised top included a small piece of extrafollicular tissue extending from a part of the periphery of the specimen. This served as a handle to avoid injury to the actual theca layer by the micro-pincette during the separation procedure.

The first method tried was to scrape off the granulosa cell layer with the tip of a fine glass needle. Examination of serial sections showed that the follicle walls treated in this way were completely free of granulosa cells. Only seldom were adherent groups of granulosa cells seen, and then only small. But follicle tops scraped in this way never survived transplantation.

Attempts to remove or facilitate removal of the granulosa cell layer with hyaluronidase proved unsuccessful with the relatively short period available between dissection and transplantation.

On the other hand, better results were obtained when the granulosa cells were washed off. The equipment used consisted of a vessel containing Krebs-Ringer-phosphate (at room temperature) and connected to a source of compressed air. The vessel was connected via a fine plastic tube to a glass tube, whose free end was drawn out to a capillary. A filter in the vessel outlet prevented passage of any impurities into the glass capillary.

The pressure with which granulosa cells were washed off was regulated by manual compression of the plastic tube. After some experience it was possible to adjust the pressure so that the granulosa could be washed off in a relatively gentle way.

Sometimes the granulosa layer appeared to be fairly firmly attached to the theca wall and therefore difficult to remove. Such follicles were always discarded. As a rule, it was possible to find some other suitable follicle in the ovary.

Corpus luteum bases

It was found that bases of corpora lutea dissected from the ovaries fairly often contained only corpus luteum cells and interstitial cells or were contaminated with only small follicles that had been found not to produce, or induce the production of, oestrogen (see Chapter X).

The bases were dissected in the following way. The apex of the corpus luteum was gripped with tweezers and all of the surface structures were cut off with micro-scissors, the incisions being carried down into the ovary. A column of corpus luteum tissue attached to the ovary only at its base was thereby obtained. The base was then cut out together with an adherent thin layer of interstitial cells, and the larger part of the corpus luteum tissue above the base was removed. These grafts were too large to be transferred by the pipette. They were carefully inserted with the pincette gripping the graft as far as possible from the base structures. These transplants were removed from ovaries in different stages of the oestrous cycle.

CONTROLS

In order to check that the response of the vaginal transplant, used as an indicator, was produced by local action of hormones from the ovarian graft, and not by steroids or their metabolites from elsewhere (*e.g.* adrenal cortex), the vagina *in situ* was regularly examined histologically, as was the piece of vaginal tissue transplanted to the other eye of the animal. This check transplant also served as a control of the behaviour of the transplanted epithelium *per se* and of the possibility that such epi-

thelium might not react in the same way as that *in situ* under the same conditions of stimulation.

The uterus was examined both macro- and microscopically, but no close analysis was made of the endometrial stroma nuclei, for example.

DURATION OF EXPERIMENT

Since the experimental period should preferably be as short as possible (see above), experiments were made to determine how soon after the transplantation oestrogen production in the ovarian grafts could be clearly recorded in the vaginal transplant used as an indicator. It was found that the transplanted vaginal epithelium reacted with pronounced growth and cornification within 7 to 8 days. Therefore an experimental period of 8 to 11 days was considered sufficient. Some experiments were of longer duration, however.

HISTOLOGICAL TECHNIQUE

The transplants were fixed in Bouin's fluid, serially sectioned (8 μ) and stained with Delafield's haematoxylin and eosin. The vagina and the uterus *in situ* were fixed in 10 per cent neutral formalin, and likewise stained with haematoxylin and eosin.

IDENTIFICATION OF OVARIAN STRUCTURES

It was, as a rule, easy to identify the various ovarian components in the stained sections of the grafts. The theca interna cells and the interstitial cells were readily distinguished from corpus luteum cells by the differences in the size of the cells and of the nuclei as well as in the appearance of the cytoplasm. The cytoplasm of the theca interna cells is more or less foamy and finely granular and thereby differs distinctly from the more homogeneous appearance of the cytoplasm of the corpus luteum cells.

In some cases — even in the short-term experiments — the ovarian grafts were found to contain altered cells difficult to

identify. Such grafts were discarded. Some of the grafts showed cellular infiltration. Since it was difficult to exclude the possibility of the grafts having undergone functional changes, such transplants were likewise discarded.

CLASSIFICATION OF OVARIAN FOLLICLES

Since it proved necessary to have a strict classification of the various types of follicles, especially for registration of the follicles contaminating the grafts, the following scheme was used in this investigation.

Type I. Primordial follicles.

Type II. Small follicles without theca interna gland cells.

Type III. Small follicles with few theca interna gland cells.

Type IV. Small follicles with well-developed theca interna gland but without antra.

Type V. Follicles with antra.

Chapter II

Behaviour of Vaginal Transplants

The behaviour of vaginal transplants was studied in some 500 preliminary or parallel experiments in addition to the material described in the present paper.

The vaginal transplants showed a very high frequency of takes. Only few transplants were seen floating freely in the aqueous humor or united to the iris simply by a thin stem at the end of the experimental period. Such grafts were always severely degenerated.

The stroma of the grafts that had healed in was of essentially the same appearance as that of the vaginal stroma *in situ*. In some cases, however, the stroma of the graft was not so rich in cells, but this did not appear to have any influence on the capacity of the epithelium to react or on its mode of reaction. Even in a few transplants with obvious, degenerative changes in the stroma, the response of the epithelium to the contiguous ovarian transplant was apparently normal.

In the majority of the vaginal grafts the epithelium showed the usual tendency of transplanted epithelia to grow out and form cysts. In unstimulated grafts this out-growing epithelium varied in appearance but was, broadly speaking, very thin except in the zone adjacent to the original epithelium, where it was frequently somewhat thicker. Its reaction to the hormones produced by the contiguous ovarian grafts varied and differed widely from that of the original epithelium. As a rule, such epithelium responded not at all or only weakly (*cf.* HILLARP and REINAND 1941), but occasionally, and then especially that part closest to the original epithelium or to the ovarian part, it reacted with the formation of a thicker epithelium with squamous or voluminous surface cells often resembling mucous cells.

Epithelium that had with certainty grown out after insertion of the transplant never underwent cornification. After the first series had shown the response of such out-growing epithelium to be atypical, no further analysis was made since it would probably not have been of any help in the evaluation of hormone production by the ovarian graft.

In the present experiments only the reaction of the original epithelium on typical stroma was considered. Vaginal grafts with cellular infiltrates were discarded because inflammatory processes are capable of inducing epithelial proliferation and even of giving rise to stratified squamous epithelium. In some cases a few leucocytes were seen here and there in the stroma and epithelium (strangely enough, they were most common in the out-growing epithelium) without any demonstrable relation to hormonal influence. The leucocyte invasion of the vaginal mucosa in met- and dioestrus is most likely not directly related to hormonal stimuli. In animals with normal cycles vaginal transplants show the same picture as the epithelium of the vagina *in situ* in the various cycle phases but never any leucocytes in met- and dioestrus (HILLARP and REINAND 1941). Another observation suggesting that factors other than purely hormonal stimuli are responsible for the leucocyte infiltration is that such cells are very scanty in smears taken from normally cycling germ-free rats in met- and dioestrus (GUSTAFSSON and SJÖVALL 1959).

Under certain conditions the original epithelium becomes thick without being influenced by hormonal stimuli. Such epithelium is often seen at the junction between original and out-growing epithelium and, secondly, there appears to be a tendency to epithelial proliferation between the original epithelium and the out-growing epithelial leaf if they are situated close together. A third source of error is that the out-growing epithelium can grow directly on the original *e.g.* in the form of a thin squamous epithelium and thus simulate a hormonal reaction. Such unspecific epithelial proliferation, *i.e.* not due to hormonal stimulation, is easily recognised and therefore did not lead to any confusion in the interpretation of the findings.

The results of the experiments confirmed that the indicator

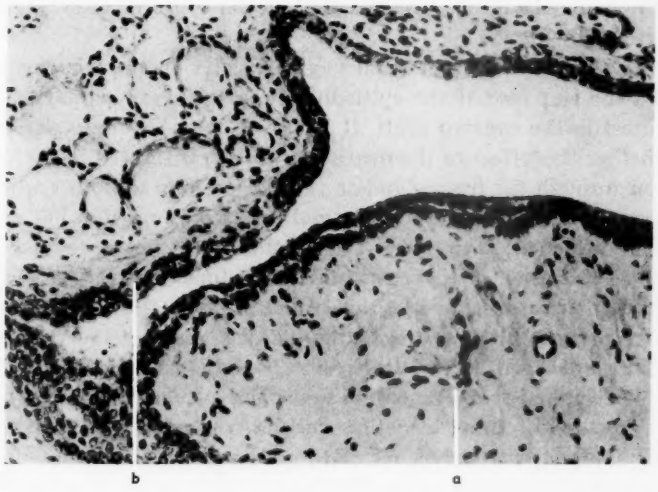


Fig. 1. Control vaginal graft (a) showing typical castrate epithelium and appearance of out-growing epithelium (b). ×200.

system used, *i.e.*, a piece of vaginal tissue contiguous to the ovarian graft, or steroids with oestrogen-like effects produced by the ovarian graft,[1] has a high sensitivity to oestrogen. Even very small aggregates of ovarian cells (such as the graft in Fig. 25 and 26) produced a marked response. This type of indicator system may be compared with the local application of oestrogen to the vaginal epithelium. It has long been known that the vaginal epithelium is very sensitive to local application of extremely small amounts of oestrogen.

The response of the epithelium to oestrogen or oestrogen-like substances often varied from one part to another of one and the same vaginal graft. When the vaginal transplant was large in relation to the ovarian graft, all types of epithelium were seen from stratified, cornified epithelium, thick squamous epithelium, in the initial phase of cornification and shedding a layer

[1] The specificity of the response of the vaginal indicator is discussed in Chapter XI.

of mucin cells, down to increasingly lower types including castrate epithelium.

As a rule, the ovarian and vaginal grafts on the iris fused, and the response of the epithelium was strongest in that part closest to the ovarian graft. It is as yet not possible to decide whether this effect of the ovarian graft is transmitted by diffusion through the tissues and/or vessels common to both grafts. Sometimes the ovarian and vaginal grafts did not fuse, but became attached to the iris at some distance from each other. When no reaction of the vaginal epithelium occurred in such cases, the transplants were discarded since the distance might have prevented the action of the ovarian graft on the vaginal transplant.

The vaginal grafts that were under the influence of oestrogen at the time of transplantation underwent cornification on the iris. This generation of cornified cells was soon desquamated and could often be seen in the vaginal cyst separated from the original epithelium by a layer of desquamated epithelial cells. It was never difficult to distinguish this cornified generation from a new one induced in the graft.

Independently of the phase of the oestrous cycle in which the vaginal graft was transplanted, it assumed the characteristics of castrate epithelium within 7 days unless stimulated by ovarian hormones. This was apparent from the control vaginal grafts in the other eye (Fig. 1) and from transplants in which the ovarian part had succumbed. This shows that transplantation *per se* does not cause unspecific epithelial changes. Neither in fairly long-term experiments carried out to assess the survival of ovarian structures did such vaginal grafts show any unspecific changes.

Unspecific epithelial reactions induced by the grafted ovarian tissue *per se* did not seem to occur either. This was shown *inter alia* from the fact that grafts containing endocrine ovarian cells of only one type did not induce any vaginal reaction.

Chapter III

Corpus Luteum Cell Transplants

As mentioned in Chapter I, cell aggregates from the interior as well as from the top of large corpora lutea were used as grafts. In most cases the latter type of graft was not contaminated or contaminated with only small follicles without theca interna cells. When corpus luteum tops were used, 2 to 5 were transplanted at a time in each experiment. This was done to check whether varying proportions of theca interna cells, possibly present in the peripheral parts of corpora lutea (see Chapter XI), might influence any oestrogen formation.

On the whole the corpus luteum grafts survived well and were of normal morphological appearance after 8 to 10 days. The grafts were always somewhat smaller than the originally transplanted pieces. This is not surprising since the grafts were relatively thick and the nutrition of some regions might have been impaired until the graft was properly vascularised, especially in grafts inserted in the anterior chamber of the eye, where practically all of the vessels grow in from one side only.

A noteworthy finding in the preliminary experiments carried out to check the survival of corpus luteum transplants was that very small pieces that had not taken on the iris but floated freely in the aqueous humor for 8 days retained their normal morphological appearance. Every other type of tissue transplanted in the present investigation rapidly degenerated under such conditions.

The behaviour of the corpus luteum grafts was studied in a series consisting of 8 transplants of cell aggregates from the interior of corpora lutea and of 16 from their top. Of these latter grafts, 4 were free of contaminants, 8 contained single small follicles without theca interna and/or primordial follicles and

Fig. 2. Large corpus luteum cell transplant (3 corpus luteum tops were transplanted together) fused with the vaginal indicator. The vaginal epithelium differs from the castrate type only by having somewhat higher mucin cells. ×80.

4 contained follicles of the types which by themselves are capable to influence the vaginal indicator (see Chapter X). All of the grafts had fused with the vaginal transplants, and especially the pieces of corpus luteum tops more or less embraced the vaginal graft. The ovarian grafts had thus a most favourable position for optimum action on the vaginal epithelium. This is illustrated in Fig. 2 showing a graft consisting of 3 corpus luteum tops from quite newly formed corpora lutea.

The epithelium of the vaginal indicator was regularly thin except in the 4 grafts contaminated with follicles containing theca interna cells (these transplants are described in Chapter X). But it differed from castrate epithelium by a somewhat greater tendency to form mucin cells than did the control vaginal grafts in the other eye. These mucin cells often were of a

somewhat higher type, never seen in the control vaginal transplant or in the vagina *in situ*. In none of the cases was any effect observed on the vagina *in situ* or the control vaginal graft. The uterus showed gross castration atrophy and no certain response microscopically.

Chapter IV

Granulosa Cell Transplants

Within 8 days the transplanted granulosa cells had developed into corpus luteum cells which were found in the grafts in aggregates of varying size and ranging from a few cells to large formations (Fig. 3). With the exception of their shape the larger aggregates were of the same appearance as the normal corpus luteum formations (Fig. 4), even regarding vascularity and connective tissue structures.[1]

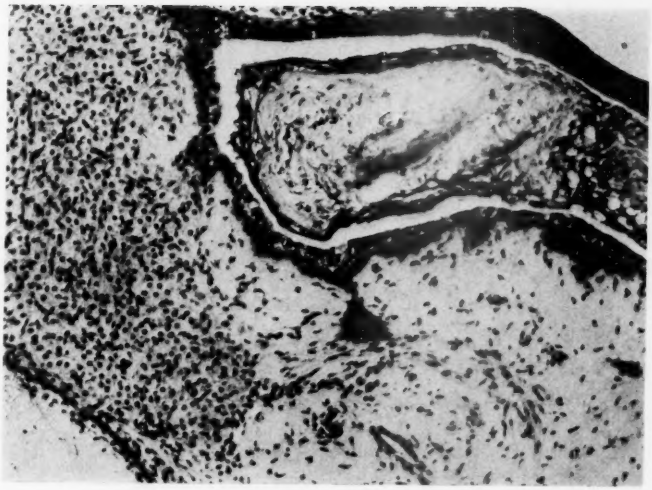
Granulosa cells that had not been luteinised could never be identified with certainty. Neither were any egg cells or rests of such cells observed in spite of the fact that often several ova were seen to accompany the granulosa cells on transplantation.

This series consisted of 10 animals. In 2, however, most of the cells flowed back out of the incision during transplantation, so that the graft consisted only of a few small aggregates of cells. In the other experiments the grafts survived with the formation of several varying sized corpora lutea in each graft, which was well fused with the vaginal graft. In the series in which uncontaminated theca interna cells were transplanted with granulosa cells (see Chapters VII and VIII), the theca interna cells succumbed in 3 of the grafts, but the granulosa cells showed a survival comparable with the 8 transplants referred to above. These 3 transplants reacted in the same way as those consisting of granulosa cells only.

The vaginal epithelium in these 13 transplants showed no reaction or possibly a very slight response consisting of a formation of higher mucin cells (see Fig. 3 and 5).

The vagina *in situ*, the control vaginal graft and the uterus showed no response.

[1] It is noteworthy that this process occurred in the absence of theca layers (*cf.* BRAMBELL 1956).



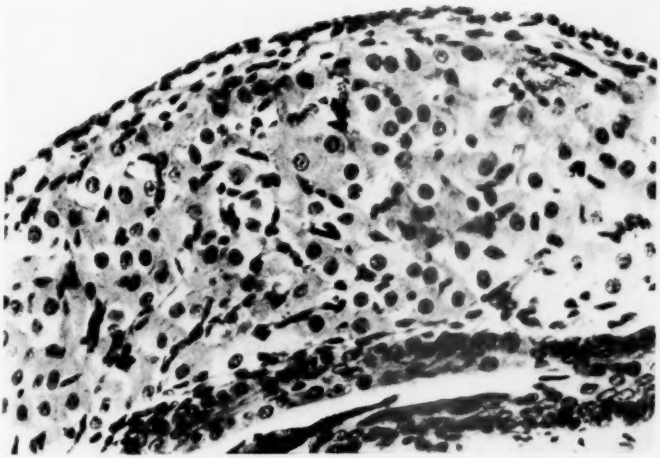


Fig. 3 and 4. Granulosa cell transplants. The granulosa cells have been luteinised and form a corpus luteum fused with the vaginal indicator. For response of the vaginal epithelium see Fig. 5. ×125 and 310.

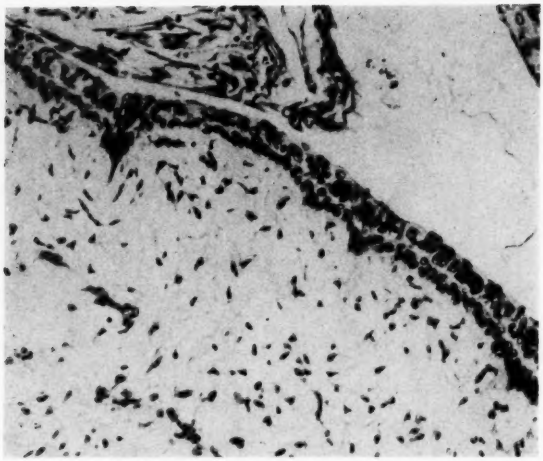


Fig. 5. Epithelium of vaginal indicator contiguous to granulosa cell transplant. It differs from the castrate type (Fig. 1) only by having somewhat higher mucin cells. ×200.

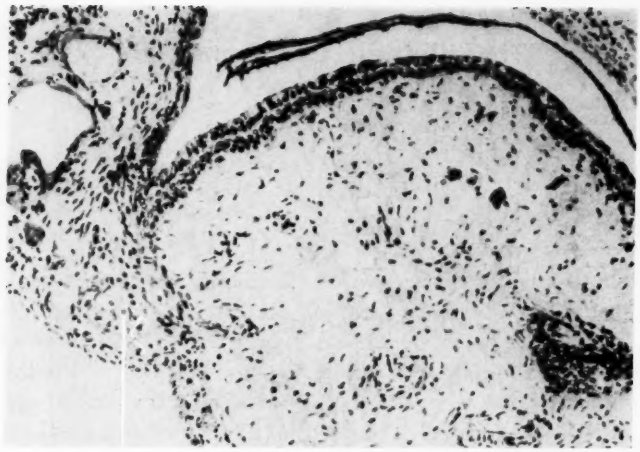
Chapter V

Theca Interna Cell Transplants

In a first series (50 animals) the granulosa cells were removed from the theca layer by scraping the follicle wall with a glass needle (see Chapter I). The technique was varied in several respects in an attempt to minimise traumatisation of the theca interna cells, but none of the grafts survived. In a smaller series the theca layer was transplanted together with granulosa cells, but — although these cells showed a good survival — the theca interna cells succumbed also in such grafts. Since, as will later be apparent, these cells are able to survive when isolated from the granulosa layer by other means, this shows how susceptible theca interna cells are to mechanical trauma.

The removal of the granulosa cells by flushing the specimens with a fine jet of fluid (Krebs-Ringer-phosphate solution) in the final experiments was much more gentle from a mechanical point of view, but nevertheless injurious, as was demonstrated by the fact that about half of the transplants contained only few or even no surviving theca interna cells. Many pycnotic or more or less shrunken cell nuclei in the ovarian graft was not an uncommon finding in these experiments.

Of 21 grafts (Table A), 11 (A 1—11) showed a good to a very good survival of theca interna cells and had fused with the vaginal indicator in a way favourable for acting upon the vaginal epithelium. Of these 11 grafts, 6 (A 1—6) contained only theca cells or theca cells contaminated with single small follicles without theca interna cells. Such small follicles have no effect *per se* on the vaginal epithelium (see Chapter X). Five (A 7—10 and 12) were contaminated with a small number of luteinised granulosa cells owing to incomplete removal of the granulosa. Such granulosa cells are transformed into corpus luteum cells



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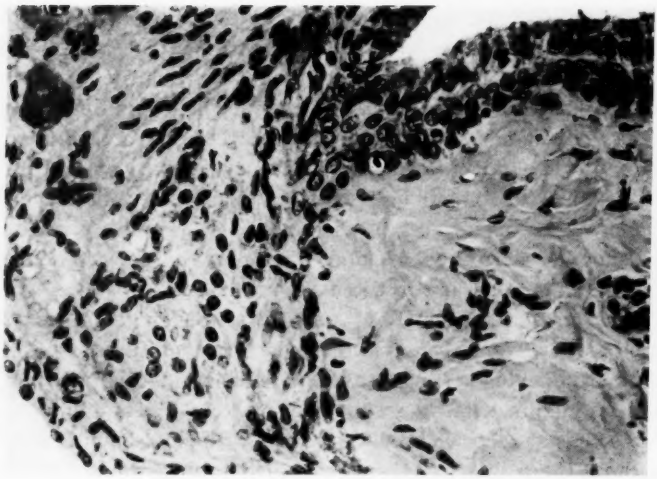


Fig. 6 and 7. Transplant of theca interna cells (A 5). The theca interna graft (a) is apparently small but has a long extension in the direction perpendicular to the section plane. In part of the vaginal epithelium the mucin cells are somewhat higher than in castrate epithelium. ×125 and 310.

a

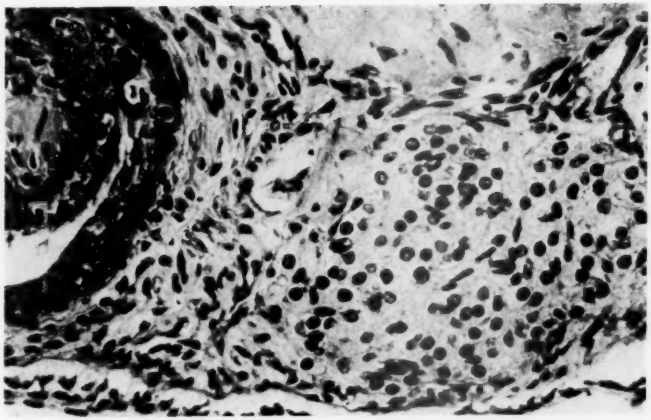
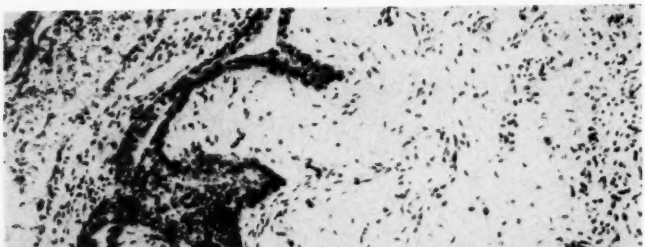


Fig. 8 and 9. Transplant of theca interna cells (A 4). Good survival of theca interna cells (a). No response of the vaginal indicator. ×125 and 310.



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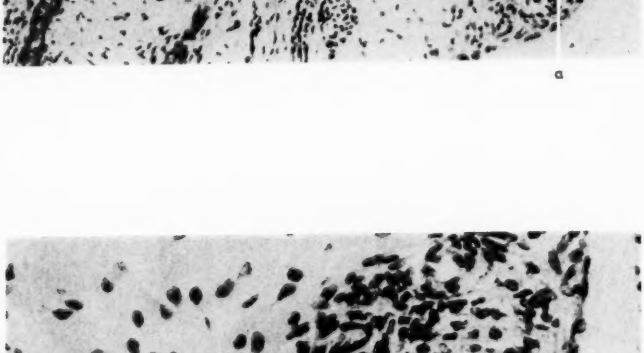
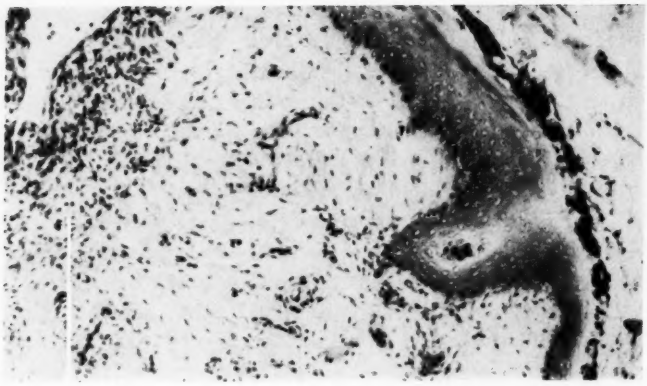


Fig. 10 and 11. Transplant of theca interna cells (A 3). For size of graft, see legend to Figs. 6—7. No response of the vaginal indicator. ×125 and 310.

within a short time (see Chapters IV and VII). Other contaminants in these 5 grafts are given in Table A.

The 6 grafts made up of theca cells only or of theca cells with single small follicles had at most a slight effect (some of the transplants showed regions with higher types of mucin cells) on the vaginal indicator (Fig. 6—11 and Table A). In another series in a related investigation in which 18 grafts were used and in which the experimental period was extended to 23—35 days, 4 showed a good survival of morphologically normal theca interna cells and no contaminants or only single small follicles without theca interna. Neither did these grafts have any demonstrable effect on the vaginal graft. The cyst content in the vaginal graft showed no signs of the ovarian graft having induced cornification during the experimental period. Microscopic examination of the control vaginal grafts as well as of the vagina *in situ* and uterus showed the picture of castration atrophy in both the short-term and the long-term experiments.

As will be shown in Chapter VII ovarian grafts containing both theca interna cells and granulosa cells had a strong oestrogen-like effect on the contiguous vaginal epithelium. The absence of any demonstrable oestrogen-like effect of the isolated theca cells might have been due to some unobservable injury despite the normal appearance of the gland cells or to the cell number of the transplants not being large enough to produce any demonstrable influence. But the grafts contaminated with granulosa cells make this explanation less likely. The survival of theca interna cells was good in 4 such grafts (A 7—10 in Table A). One of them (A 10) was not included in the following analysis because it contained follicular structures, which are by themselves capable of producing a response of the epithelium (see Chapter X). The other 3 grafts had acted upon the vaginal epithelium, which responded with the formation of thick, squamous epithelium in 2 and thick cornified epithelium in 1 (Fig. 12 and 13). Of the grafts with a poor survival of theca interna cells, one (A 12) was contaminated with granulosa cells. Though this graft contained a much smaller number of theca interna cells than grafts A 1—6, it produced a strong



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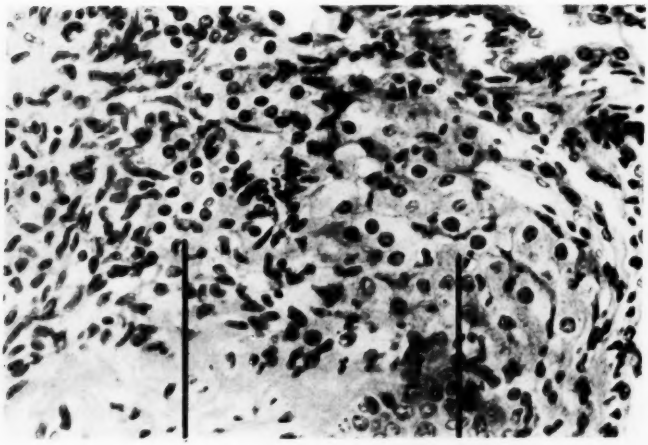
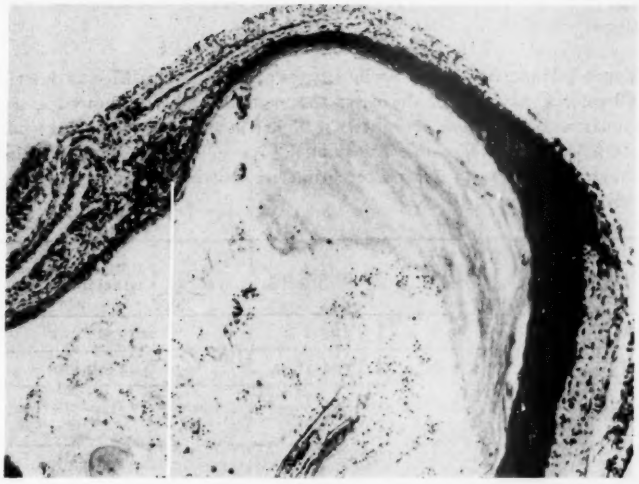


a b

Fig. 12 and 13. Transplant (A 8) of theca interna cells (a) contaminated with a small number of luteinised granulosa cells (b). The vaginal indicator has responded with formation of thick cornified epithelium. ×125 and 310.



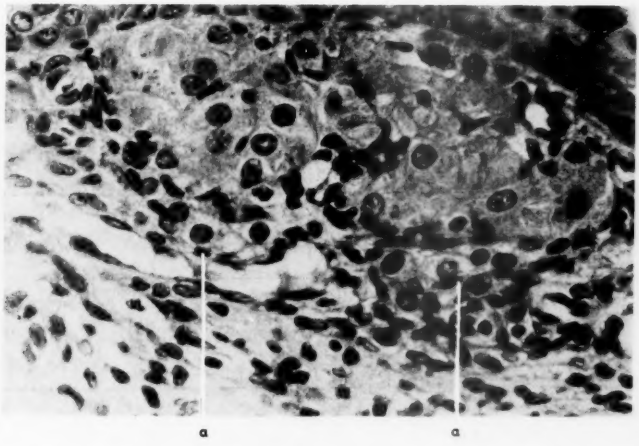


Fig. 14 and 15. Transplant of theca interna cells (A 12) contaminated with a small number of luteinised granulosa cells. The ovarian graft (a in Fig. 14) is very small, contains only few theca interna cells (a in Fig. 15) and is situated in the wall of the vaginal cyst, but the vaginal indicator has nevertheless responded with formation of thick cornified epithelium. ×80 and 500.

TABLE A

Transplants of theca interna cells

Types I, II etc. denote the various types of follicles classified as described in Chapter I. In some of the cases the vaginal indicator showed a slight response with formation of somewhat higher mucin cells, but this response is classified as "no reaction". The symbol "+ + +" denotes survival of large numbers of apparently normal theca interna cells in the grafts and "0", no survival.

No.	Experimental period in days	Survival of theca interna cells	Contaminants	Epithelium of vaginal indicator
A 1	8	+ + +	1 Type I	No reaction
A 2	8	+ + +	2 Type II	No reaction
A 3	10	+ + +	1 Type I 3 „ II	No reaction
A 4	10	+ + +	None	No reaction
A 5	10	+ + +	None	No reaction
A 6	10	+ + +	1 Type I 2 „ II	No reaction
A 7	8	+ +	Small numbers of corpus luteum cells No follicles	Thick stratified mucified
A 8	8	+ +	Small numbers of corpus luteum cells No follicles	Cornified
A 9	10	+ + +	Few corpus luteum cells 1 Type I	Thick stratified squamous
A 10	10	+ + +	Small numbers of corpus luteum cells 8 Type I 1 „ III	Thick stratified mucified
A 11	10	+ + +	1 Type IV	Cornified
A 12	11	+	Small numbers of corpus luteum cells 3 Type II	Cornified
A 13	7	0	1 Type I	No reaction
A 14	8	+	1 Type III	4 layered

A 15	8	0	None	Outgrowing epithelium directly on the original epithelium. This is probably 2—3 layered
A 16	8	+	2 Type I 1 „ II 1 „ III	4—6 layered squamous
A 17	8	+	3 Type I 1 „ II	No reaction
A 18	10	+	1 Type I 1 „ II 1 „ III	No reaction
A 19	11	+	None	No reaction
A 20	10	+	2 Type I 1 „ II 1 „ III	No reaction
A 21	10	+	1 Type I	No reaction

response of the vaginal graft, which developed thick squamous epithelium with marked cornification (Fig. 14 and 15). The lack of a regular correlation between the number of surviving cells and the response of the vaginal epithelium, as in this series, is discussed in Chapter XI.

Observations made in experiments with follicle walls from which the granulosa was first removed and the theca then transplanted together with granulosa or corpus luteum cells (Chapter VIII) also strongly argue against the absence of any oestrogenic effect of the isolated theca interna cells being due to possible injury of these cells.

Chapter VI

Interstitial Cell Transplants

In this series, which consisted of 16 animals (B 1—16 in Table B) the interstitial cells used were taken from the central part of the ovary. The grafts were composed of a small single gland lobe or in some of the cases of two or more adherent lobes. In all of the cases the survival was good, and even the smallest transplants (see Fig. 16, 20 and 21) contained a much larger number of cells than those theca cell transplants (see Chapter V), that showed the best survival in their group. Some of the interstitial cell transplants were very large, as is apparent from Fig. 17—19, which illustrate the largest graft (B 6) in this series.

Of the 16 grafts, 5 (B 1—5) were made up of interstitial cells only, 3 (B 6—8) were contaminated with small follicles without theca interna cells and the remaining 8 (B 9—16) were contaminated with varying sized follicles with theca interna.

In all of the experiments the ovarian part had fused with the graft in a way favourable for action on the vaginal epithelium.

In all of the transplants (B 1—8) in which the ovarian grafts contained only interstitial cells or consisted of interstitial cells and small follicles without theca interna, the contiguous vaginal grafts showed no epithelial reaction or, if so, it was only slight with the formation of higher types of mucin cells in parts of some of the grafts. The other grafts (B 9—16) regularly induced an epithelial proliferation, usually with the development of thick cornified epithelium. Fig. 22 shows such a graft (B 12) which was contaminated with a single follicle with beginning antrum formation which induced a pronounced cornification of the vaginal epithelium in spite of the fact that it did not contain so many interstitial cells as graft B 3 (Fig. 16), which pro-

TABLE B

Transplants of interstitial gland cells

Types I, II etc. denote the various types of follicles classified as described in Chapter I. In some of the cases the vaginal indicator showed a slight response with formation of somewhat higher mucin cells but this response is classified as "no reaction".

No.	Experimental period in days	Contaminants	Epithelium of vaginal indicator
B 1	10	None	No reaction
B 2	10	None	No reaction
B 3	10	None	No reaction
B 4	10	None	No reaction
B 5	10	None	No reaction
B 6	10	25 Type I 10 „ II	No reaction
B 7	10	1 Type II	No reaction
B 8	10	2 Type I 1 „ II	No reaction
B 9	10	1 Type I 2 „ II 2 „ III	Thick stratified mucified
B 10	10	1 Type II 2 „ V	Cornified
B 11	11	5 Type I 13 „ II 3 „ III 2 „ IV	3—4 layered
B 12	10	1 Type V	Cornified
B 13	10	2 Type II 1 „ III 2 „ V	Cornified
B 14	10	8 Type II 3 „ III	Cornified
B 15	10	1 Type I 1 „ II 1 „ IV	Cornified
B 16	10	2 Type II 1 „ III 1 „ IV 2 „ V	Cornified

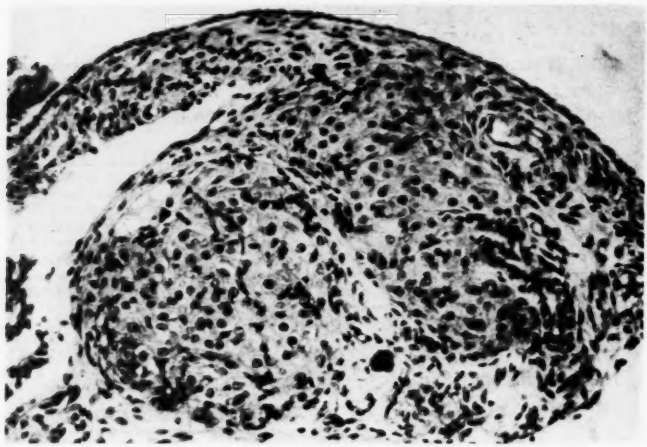
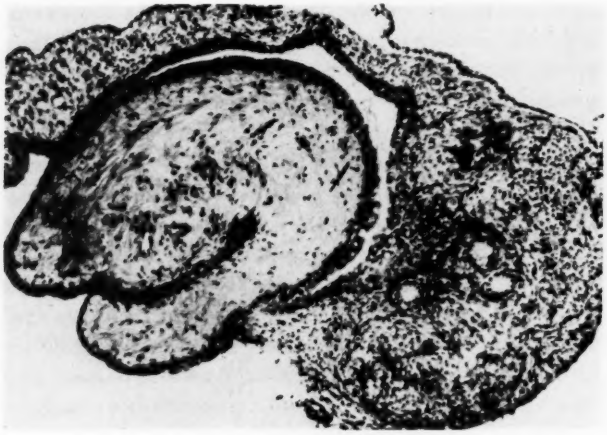


Fig. 16. Transplant of interstitial cells (B 3) showing good survival of the cells. ×200.

duced no response at all, though it had likewise fused with the vaginal graft. The series thus included very large interstitial cell transplants containing no follicles with theca interna, which produced no response of the vaginal graft, and secondly,



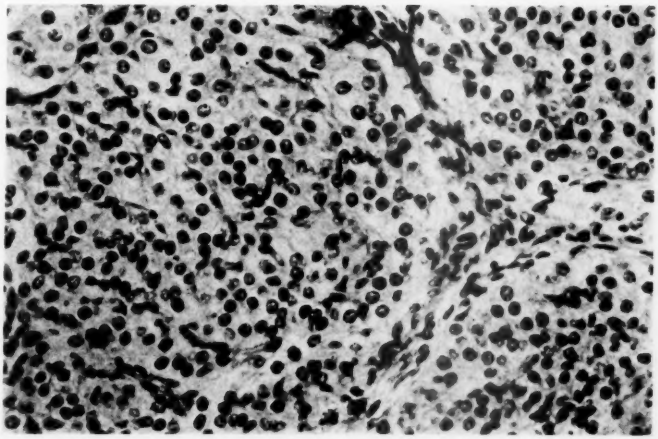


Fig. 17—19. Large transplant (B 6) of interstitial cells contaminated with follicles of Types I and II. The ovarian graft (Fig. 18 and 19) shows good survival of several gland lobes. Part of the vaginal epithelium has somewhat higher mucin cells than the castrate type. ×76, 125 and 310.

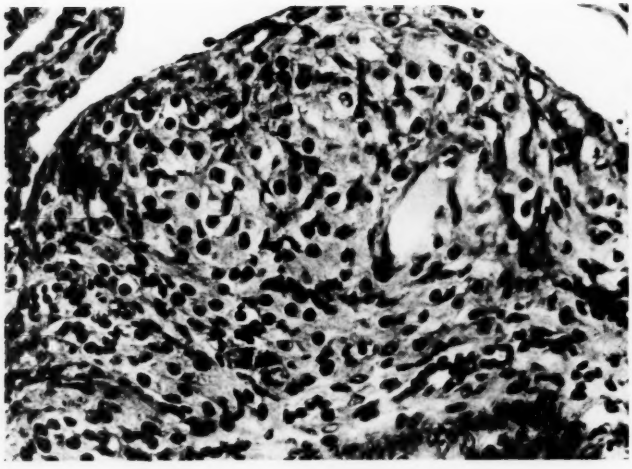


Fig. 20 and 21. Transplant of interstitial cells (B 7). Response of the vaginal indicator as in Fig. 17—19. ×125 and 310.

Fig. 22. Transplant of interstitial cells (B 12) contaminated with one small Type V follicle. Vaginal epithelium heavily cornified. ×125.

interstitial cell grafts contaminated by follicles with theca interna, which even when the number of interstitial cells was small, regularly caused a reaction of the epithelium.

In one series (not accounted for in Table B) used for a purpose beyond the scope of the present investigation interstitial cells were transplanted to animals treated with gonadotrophic hormones. The experimental period was 10 days and on the 7th, 8th and 9th day the animals received a daily subcutaneous injection of 5 I.U. of human chorionic gonadotrophin or pregnant mare serum gonadotrophin. Three of the grafts contained abundant surviving gland cells of morphologically normal appearance without contaminants, but they did not produce any reaction of the epithelium of the vaginal indicator. On the other hand, the ovarian grafts contaminated by follicles with theca interna produced a marked reaction of the vaginal epithelium which became thick and cornified.

In none of the above experiments, with the exception of B 13 (see Chapter X), did the ovarian graft have any demonstrable effect on the vagina or uterus *in situ* or on the control vaginal transplant in the other eye.

Chapter VII

Follicle Wall Transplants

This series consisted of 27 experiments. In 2 the vaginal graft succumbed, in 3 (Table C, 23—25) the ovarian graft was contaminated with small follicles with theca interna, which are able *per se* to influence the vaginal transplant (see Chapter X). Of the remaining 22 grafts (Table C, 1—22), 9 were not contaminated at all, while the others contained small follicles which are not able by themselves to produce any reaction of the vaginal graft (see Chapter X).

The survival of the theca interna cells was generally good, and most of the grafts were found to contain a relatively large number of morphologically normal cells. This was expected because the method used is gentle and the traumatisation of the cells therefore minimal. The wide variation in the number of cells in the different grafts (Table C) was probably due to corresponding differences in the size of grafts implanted. This was in turn due to the fact that in some of the ovaries all of the large follicles were seated somewhat deeper so that only a small fragment of thin follicle wall could be excised. In 3 experiments (C 1, 13 and 14) pieces of the wall from smaller follicles with antra were transplanted. The amount of theca interna cells and luteinised granulosa cells in these grafts (Fig. 27 and 28) is comparable to that in most of the other grafts and the cells also behaved in the same way.

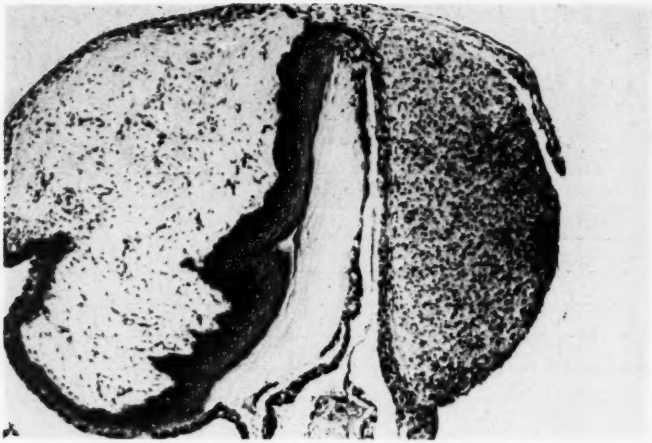
The granulosa cells developed into corpus luteum cells in the same way as in grafts with granulosa cells only, and when abundant they also formed microscopically typical corpus luteum formations. In some experiments (C 13, 18, 20 and 21) the graft was found to contain typical granulosa cells intermingled with the corpus luteum cells.

TABLE C

Transplants of follicle wall

For explanation of "Types I, II" etc. and the survival of the theca interna cells, see Table A.

No.	Experimental period in days	Survival of theca interna cells	Contaminants	Epithelium of vaginal indicator
C 1	15	+ + +	None	Cornified
C 2	8	+ + +	None	Cornified
C 3	9	+ +	None	Cornified
C 4	9	+ + +	None	Cornified
C 5	8	+ +	None	Cornified
C 6	8	+ +	None	Cornified
C 7	9	+	None	Cornified
C 8	8	Few	None	4—5 layered
C 9	8	+	7 Type I	4—5 layered mucified
C 10	10	0	2 Type I	No reaction
C 11	14	0	No granulosa or corpus luteum cells. No contaminants	No reaction
C 12	7	0	No granulosa or corpus luteum cells. No contaminants	No reaction
C 13	15	+ +	1 Type I	Cornified
C 14	14	+ +	3 Type I	Cornified
C 15	8	+ +	2 Type I 3 „ II	Cornified
C 16	8	+ +	1 Type I	Cornified
C 17	8	+ + +	10 Type I	Cornified
C 18	8	+ + +	2 Type I	Cornified
C 19	9	+ + +	4 Type I	Cornified
C 20	8	+ +	1 Type I	Cornified
C 21	8	+ +	None	Cornified
C 22	11	+ + +	4 Type I 2 „ II	Cornified
C 23	14	+	3 Type II 1 „ IV	Cornified
C 24	8	+ +	1 Type IV	Cornified
C 25	10	+	1 Type IV	Cornified



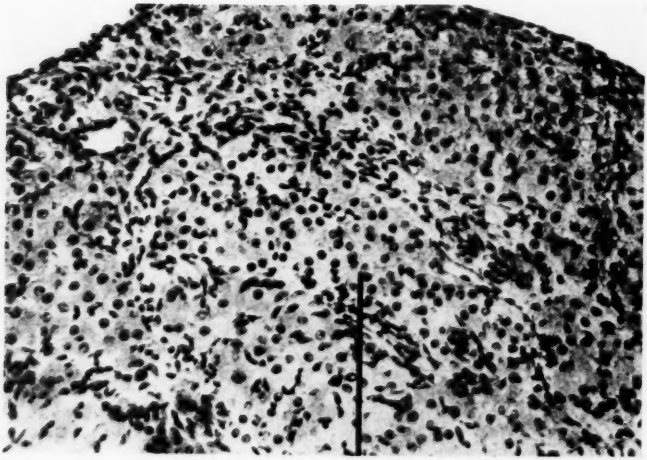
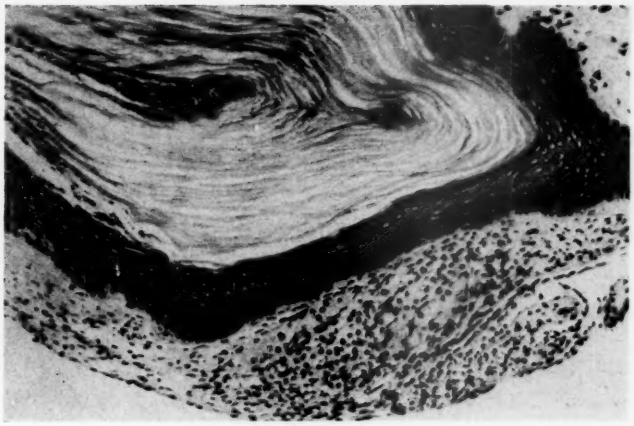


Fig. 23 and 24. Transplant of follicle wall (C 4). Good survival of theca interna cells (a) enveloped by luteinised granulosa cells. Vaginal indicator heavily cornified. ×80 and 200.



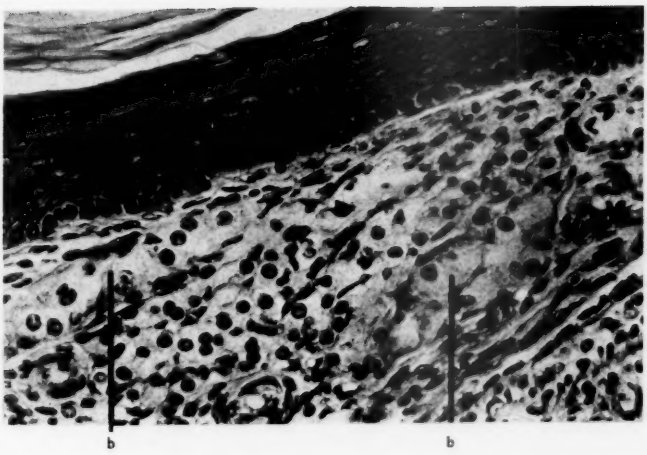
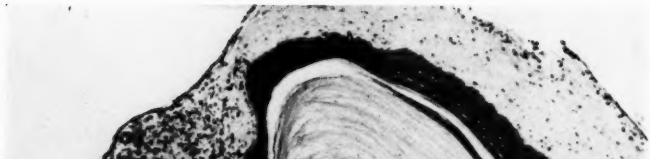


Fig. 25 and 26. Transplant of follicle wall (C 16). The ovarian graft is rather small and contains only few luteinised granulosa cells (b) surrounding theca interna cells. Vaginal indicator heavily cornified. ×125 and 310.



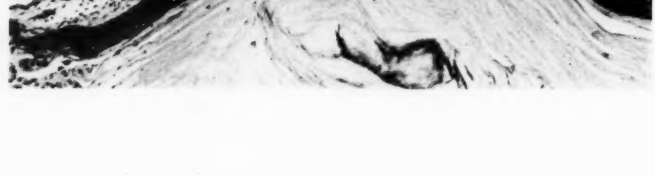


Fig. 27 and 28. Transplant of follicle wall (C 13). The ovarian graft contains theca interna cells (a) and luteinised granulosa cells (b). Vaginal indicator heavily cornified. ×80 and 310.

In some cases a relatively large portion of the cupola of the follicle could be extirpated. These grafts showed a tendency to turn inside out with the result that the theca interna cells were found in a hilus-like formation enveloped by corpus luteum cells (Fig. 24).

The vaginal transplants fused with the ovarian grafts showed — with 5 exceptions — a pronounced response with formation of a thick cornified epithelium of the same appearance as that produced by oestrogens.

Of these 5 exceptions, the follicle wall graft had succumbed in 2 (C 11 and 12). The other experiments deserve commentation.

In 1 animal (C 10), in which the vaginal transplant showed no reaction at all, it was found that no theca interna cells had survived. The granulosa cells had, however, developed a fairly large corpus luteum formation of morphologically normal appearance. This graft thus behaved in essentially the same way as transplants consisting of isolated granulosa cells. In the other two cases (C 8 and 9) the vaginal transplant showed only slight epithelial growth. These transplants contained abundant corpus luteum cells, but fewer theca interna cells than any other transplant in this series.

One graft (C 16, Fig. 25 and 26) contained only few corpus luteum cells, but nevertheless caused cornification of the vaginal transplant.

The cornification of the vaginal graft was often pronounced (Fig. 25), and in 3 cases in which the experiment was allowed to proceed somewhat longer (C 1, 13, 14) it seems to have been continuous since a continuous mass of horny lamellae was seen outside of the actual cornified epithelium (Fig. 27).

In no instance was the graft found to have any effect on the vagina or uterus *in situ* or on the control vaginal transplant in the other eye.

In a series of 23—35 day experiments grafts containing no contaminating follicles also induced cornification of the contiguous vaginal epithelium. But these grafts were found to contain abundant epithelioid cells, whose nature could not be identified with certainty: they are therefore not included in Table C.

Chapter VIII

Theca Interna Cell Transplants Combined with Granulosa Cells or Corpus Luteum Cells

Transplants containing both theca interna cells and granulosa cells exert a strong oestrogen-like effect on the contiguous vaginal indicator (see Chapter VII), while neither type of cell can by itself produce any such effect (see Chapters IV and V). Although it is tempting to assume that the presence of both types of cells in combination is necessary for the production of any such effect, a possibility that should be borne in mind is that the cells, especially the theca interna cells, might have been functionally damaged during their removal and/or transplantation despite their normal appearance in the graft. The probability of such functional impairment is, however, diminished by the observations set forth in Chapter V.

In order to check these two possibilities, theca interna cells were freed from granulosa cells by the same method as that used in the preparation of the theca cell transplants accounted for in Chapter V. They were then transplanted together with granulosa cells obtained in the way described (page 12). In 1 case (D 1 in Table D; Fig. 29 and 30) theca interna cells were transplanted together with a cell aggregate from a large corpus luteum.

The series consisted of 12 transplants (D 1—12 in Table D). Of these, no ovarian structures at all were found in 1 case (D 11), and in 2 cases (D 5 and 10) no theca interna cells survived. But in these 2 cases the graft was found to contain abundant luteinised granulosa cells. In none of these 3 cases did the contiguous vaginal epithelium show any response. Another case (D 12) showed 2 small follicles with theca interna cells. That transplant also contained a few corpus luteum cells in direct contact with theca interna cells, so that it could not be excluded

TABLE D

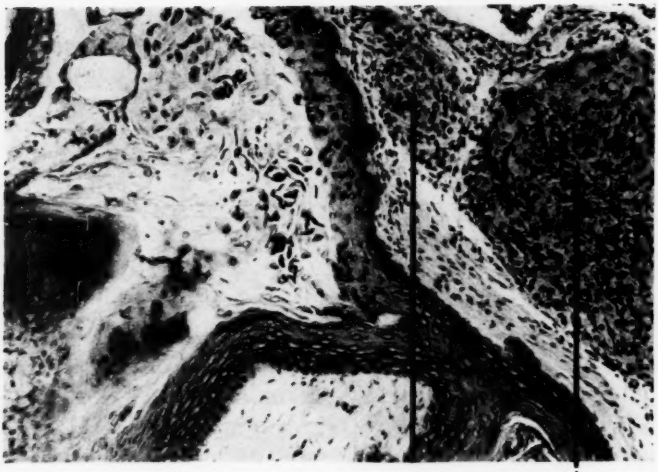
Transplants of theca interna cells combined with granulosa cells

For explanation of "Types I, II" etc. and the survival of the theca interna cells, see Table A.

No.	Experimental time in days	Survival of theca interna cells	Contaminants	Epithelium of vaginal indicator
D 1	10	+ + +	None	Cornified
D 2	10	+ + +	None	7—8 layered mucified
D 3	10	+ +	None	Cornified
D 4	10	+ +	None	Cornified
D 5	11	0	None	No reaction
D 6	11	+ + +	3 Type I	Cornified
D 7	15	+ +	2 Type I 2 „ II	Cornified
D 8	10	+	5 Type I 4 „ II	4 layered mucified
D 9	10	Very few	2 Type I 1 „ II	No reaction
D 10	10	0	2 Type I 1 „ II	No reaction
D 11	10	0	2 Type II No granulosa cells survived	No reaction
D 12	10	+ + +	Few corpus luteum cells 3 Type II 2 „ III	Cornified

that the transplanted follicle cupola had been deprived of all adherent granulosa cells. The vaginal indicator had responded with cornification.

The remaining 8 transplants (D 1—4 and 6—9) were not contaminated or, if so, only by very small follicles without theca interna, which cannot by themselves exert any demonstrable influence on the vaginal epithelium of the graft (Chapter X). These 8 grafts contained theca interna cells and abun-



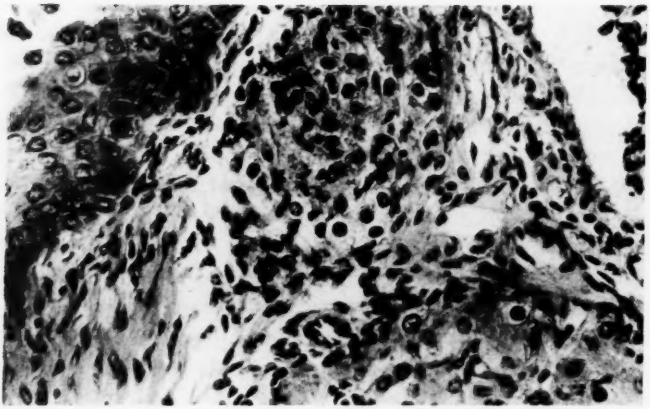
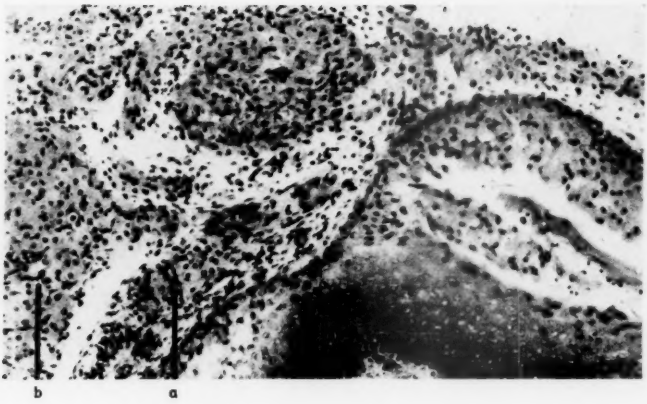


Fig. 29 and 30. Transplant (D 1) of theca interna cells (a) combined with corpus luteum cells (b). The vaginal indicator has responded with formation of thick cornified epithelium. The epithelium seen in Fig. 30 is a part of the out-growing vaginal epithelium. ×125 and 310.



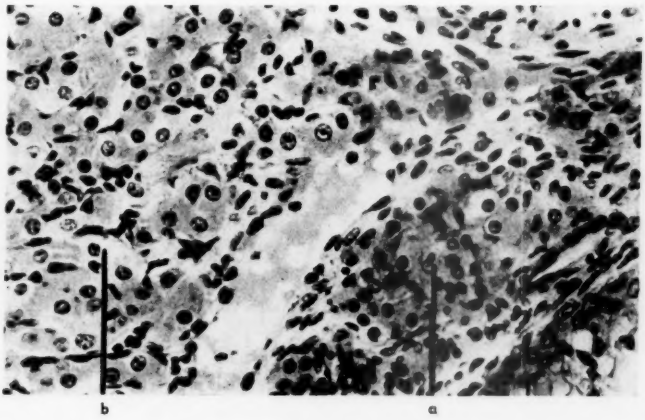
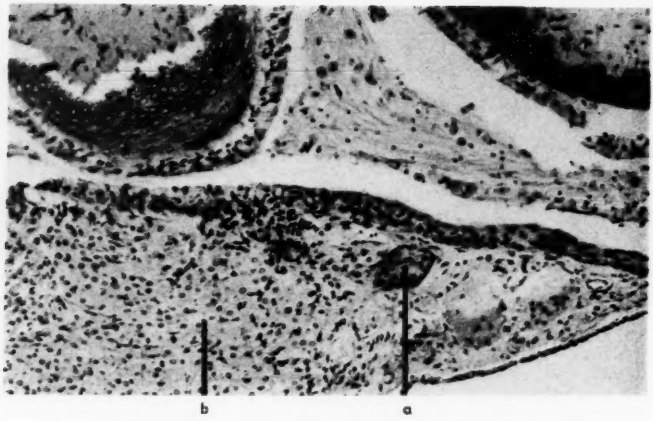


Fig. 31 and 32. Transplant (D 3) of theca interna cells (a) combined with granulosa cells (b). The granulosa cells have been luteinised. Response of vaginal indicator as in Fig. 29—30. $\times$125 and 310.



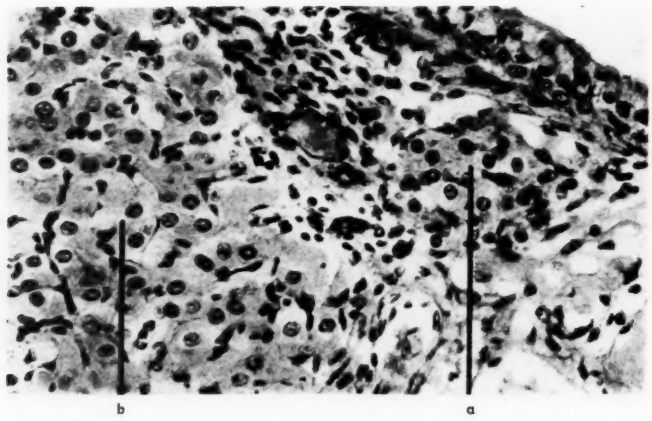
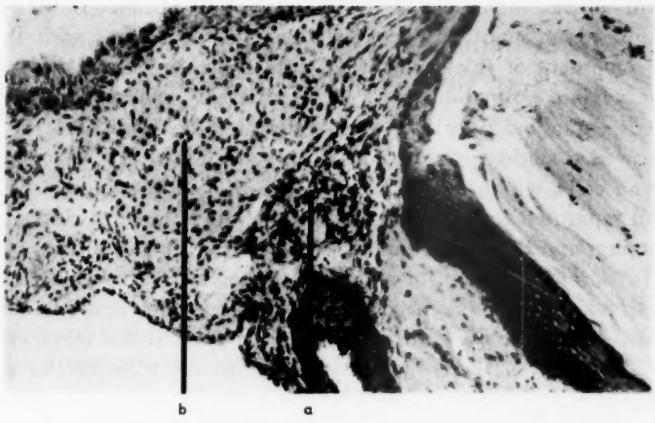


Fig. 33 and 34. Transplant (D 4) of theca interna cells (a) combined with granulosa cells (b). See legend to Fig. 31—32.



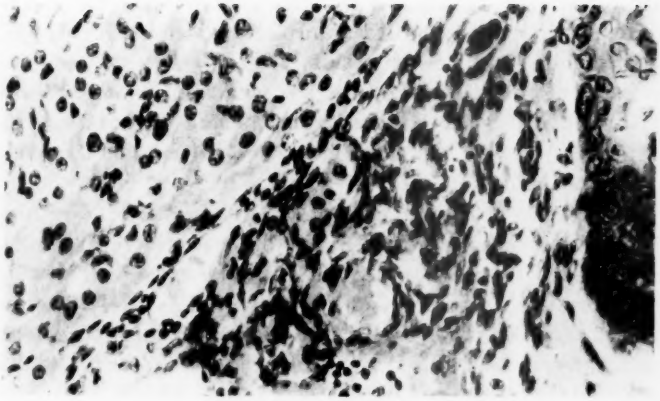


Fig. 35 and 36. Transplant (D 7) of theca interna cells (a) combined with granulosa cells (b). See legend to Fig. 31—32.

dant corpus luteum cells. With but one exception (D 9) the vaginal transplant had regularly reacted, in 5 cases with the development of thick, cornified epithelium. In transplant D 9 the vaginal epithelium showed no signs of reaction, but that graft contained only few theca interna cells. Transplant D 8 showed a slight reaction, but it only contained few theca interna cells, which were lodged in the wall of the vaginal cyst and separated from the original epithelium by outgrowing epithelium and the cyst lumen. This ovarian graft was thus not in a favourable position for acting upon the vaginal indicator.

In no instance was any effect observed on the vagina or uterus *in situ* or on the control vaginal transplant in the other eye.

The series of theca interna cell transplants (Chapter V) included 6 (A 1—6 in Table A) with good survival but without any demonstrable reaction of the contiguous vaginal epithelium. In the present series there were 7 transplants (D 1—4 and 6—8) that produced a reaction of the vaginal epithelium. These transplants had no contaminants capable of acting upon the vaginal epithelium. Four of these transplants are illustrated in Fig. 29—36 for comparison with the reproductions of uncontaminated theca interna cell transplants given in Fig. 6—11. The technique applied for washing off the granulosa cells was the same in both series, and there is no reason to suppose any systematic difference in the actual technical procedure. In transplants A 1—6 the theca interna cells showed the same appearance and their survival was equally good, if not better, than in the above mentioned 7 transplants in this series, which also argues against any systematic difference in the technical performance of the experiment. These observations together with those set forth in Chapter V make it less likely that the lack of response of the vaginal epithelium to uncontaminated theca interna cells was due to any supposed damage to the latter.

Chapter IX

Interstitial Cell Transplants Combined with Corpus Luteum Cells

The experiments described in Chapter VI showed that the ovarian grafts consisting solely of interstitial cells produced no reaction of the contiguous vaginal epithelium. In analogy with the investigation of the theca interna cells with and without granulosa cells (corpus luteum cells) ovarian grafts containing both interstitial cells and corpus luteum cells were studied for any oestrogenic effect on the vaginal indicator. As described above (page 14) such grafts can be secured by transplanting corpus luteum bases with a thin layer of the underlying ovarian tissue.

It is not possible to excise the corpus luteum bases without distorting underlying interstitial cells. Such distortion manifested itself in many of the grafts by the occurrence of abundant changed cells and shrunken or pycnotic nuclei. The interstitial cells had obviously been traumatised much more severely than those found in the transplants consisting of interstitial cell lobes (Chapter VI).

Every transplant, however, contained morphologically normal cells varying widely in number from very few to abundant (see Table E).

The corpus luteum cells survived well, and every transplant was found to contain a small to large corpus luteum formation with typical cells.

A disadvantage of this type of transplant is that it is difficult to avoid contaminating follicles. Of 22 transplants (Table E, 1—22) three (E 7, 10 and 12) were uncontaminated, 9 (E 1—6, 8, 9 and 11) were contaminated by small follicles without theca interna, which are by themselves unable to influence the vaginal epithelium, and 10 (E 13—22) were contaminated by fol-

TABLE E

Transplants of corpus luteum bases with adherent interstitial cells

For explanation of "Types I, II" etc. and the survival of interstitial cells, see Table A.

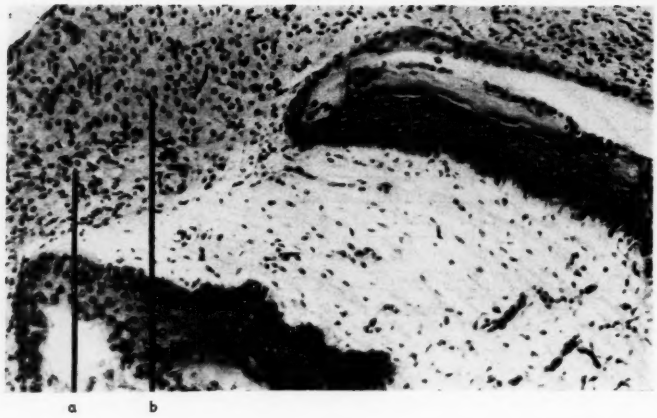
No.	Experimental time in days	Survival of interstitial cells	Contaminants	Epithelium of vaginal indicator
E 1	8	+	4 Type I	No reaction
E 2	10	+	2 Type II	3—4 layered
E 3	10	Very few	1 Type II	No reaction
E 4	10	+	5 Type I 8 „ II	4—5 layered
E 5	9	+ +	3 Type I	5—6 layered mucified
E 6	8	+ +	5 Type I 5 „ II	Thick stratified squamous
E 7	10	+ +	None	Thick stratified squamous
E 8	10	+ +	6 Type I 4 „ II	Cornified
E 9	10	+ +	1 Type II	Cornified
E 10	10	+ +	None	Cornified
E 11	11	+ + +	3 Type I 2 „ II	Cornified
E 12	10	+ + +	None	Cornified
E 13	8	+	1 Type IV	Cornified
E 14	9	Very few	1 Type V	Cornified
E 15	9	+ + +	1 Type II 1 „ IV	Cornified
E 16	10	+	1 Type V	Cornified
E 17	10	+ + +	4 Type I 5 „ II 1 „ III	4—6 layered stratified squamous and mucified
E 18	10	+	3 Type I 5 „ II 1 „ III 1 „ IV	Cornified

E 19	10	+ +	2 Type I 1 „ II 1 „ IV	Cornified
E 20	11	+ + +	2 Type I 1 „ III 1 „ V	5—6 layered mucified
E 21	10	abundant	7 Type I 1 „ II 2 „ III 1 „ V	Cornified
E 22	10	abundant	7 Type I 9 „ II 1 „ III 1 „ V	Cornified

licles with theca interna. The relative number of transplants free from follicles together with those contaminated by only small follicles without theca interna was roughly the same as in the transplants of interstitial cells described in Chapter VI, but the relative number of transplants completely free of such contaminants was much smaller. This difference may be explained by the fact that the follicles are more scanty in the central part of the ovary.

In the excised corpus luteum base the interstitial cell aggregates appeared as very small white lobes, and the amount transplanted was always small except in 2 cases (E 21 and 22). The number of morphologically normal cells in all of the transplants except these 2 was smaller, often much smaller, than the amount of cells in the interstitial cell lobe transplants (compare Fig. 37—42 with 16—21). It hardly ever exceeded the amount of theca interna cells in those transplants (Chapter V) that showed the best survival in this group.

Despite this difference in the amount of surviving interstitial cells and in contradistinction to the grafts containing only interstitial cells the grafts containing also corpus luteum cells produced a reaction of the vaginal indicator. This type of graft was found to behave like follicle wall transplants and exert an



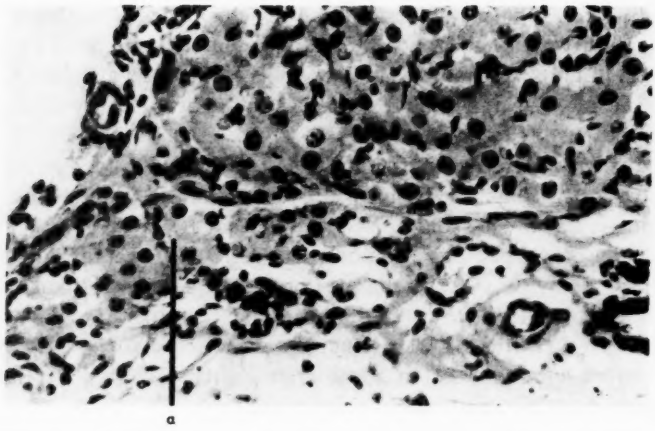
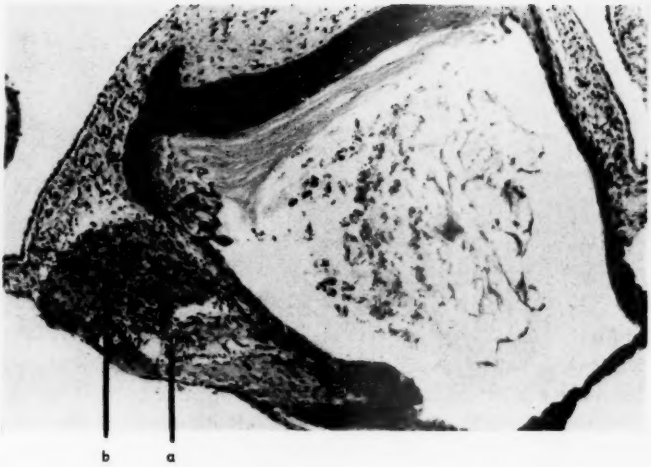


Fig. 37 and 38. Transplant (E 12) of a corpus luteum base (b) with adherent interstitial cells (a). Vaginal indicator with thick cornified epithelium. ×125 and 310.



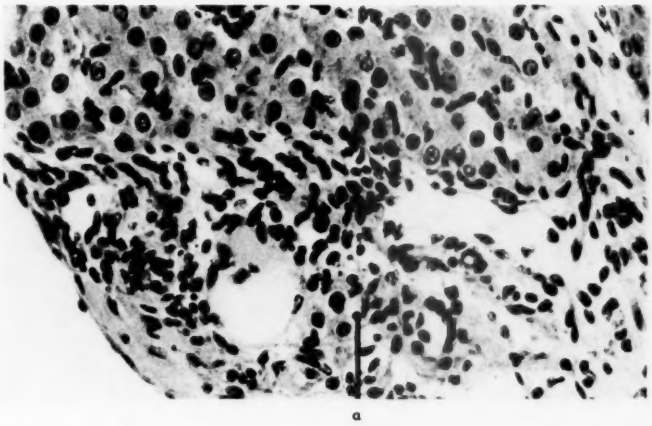
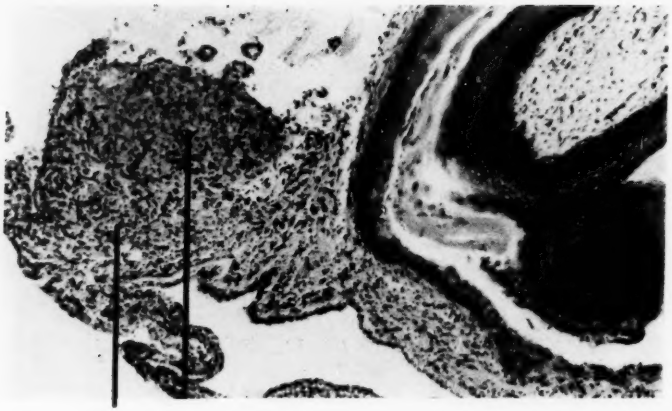


Fig. 39 and 40. Transplant (E 10) of a corpus luteum base (b) with adherent interstitial cells (a). Vaginal indicator with thick cornified epithelium. ×80 and 310.



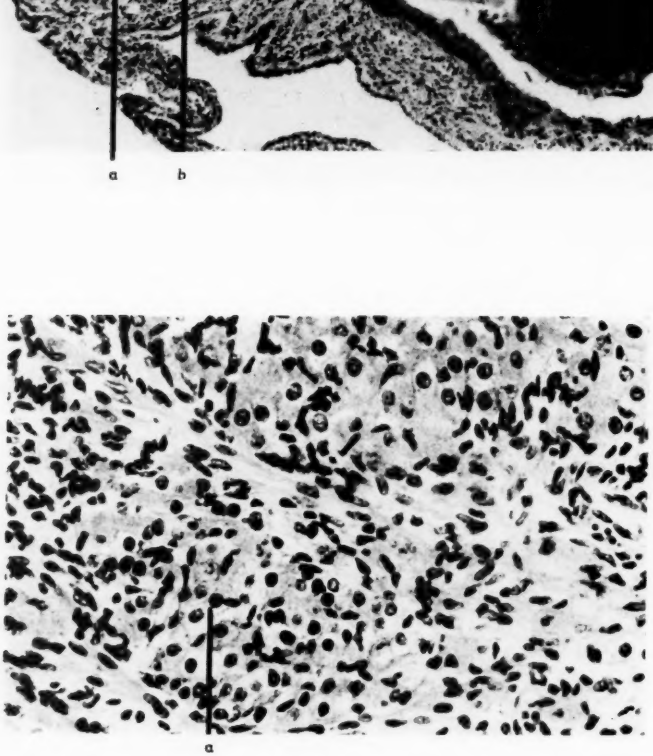


Fig. 41 and 42. Transplant (E 11) of a corpus luteum base (b) with adherent interstitial cells (a). See legend to Fig. 39—40.

oestrogen-like effect on the vaginal epithelium (Fig. 37, 39 and 41).

In the present series, in which the number of morphologically normal interstitial cells differed widely from one transplant to another, the reaction of the vaginal epithelium was found to vary with the number of such cells. Of the transplants not contaminated or contaminated only by small follicles without theca interna (E 1—12), those (E 1—4) with the smallest amount of interstitial cells produced no reaction or a slight proliferation of the epithelium, while transplants with more abundant interstitial cells produced a strong response of the epithelium with the formation of thick squamous epithelium in 2 cases and cornification of such thick epithelium in 5. One transplant (E 5) with a relatively large number of interstitial cells produced a weaker reaction with the formation of 5—6 layered mucified epithelium.

In no instance did the grafts have any demonstrable effect on the vagina or uterus *in situ* or on the control vaginal transplant in the other eye.

In transplants E 1—12 there thus appeared to exist a correlation between the number of interstitial cells and the strength of the response of the vaginal epithelium. Since all of these transplants contained a fair amount of corpus luteum tissue, the latter alone, like the corpus luteum tops, did not seem to be able to produce any response of the indicator.

In order further to check that the combination of interstitial cells and corpus luteum cells is necessary for the production of the oestrogen-like effect in these transplants the following experiments were performed.

Cell aggregates of the interstitial gland from the central part of the ovary were transplanted in combination with interior aggregates or tops (one in each experiment) from large corpora lutea. The transplants thus consisted of a combination of two ovarian cell systems, neither by itself capable of producing a response of a contiguous vaginal graft (see Chapters III and VI).

In each experiment only one very small interstitial gland lobe or a part of a larger one was dissected to secure transplants which, in contrast to the transplants B 1—8 in Chapter VI, con-

TABLE F

Transplants of interstitial cells combined with corpus luteum cells

For explanation of "Types I, II" etc. and the survival of interstitial cells, see Table A.

No.	Experimental time in days	Survival of interstitial cells	Contaminants	Epithelium of vaginal indicator
F 1	8	+	None	3—4 layered
F 2	8	+ +	1 Type II	Cornified
F 3	8	+ +	2 Type I 1 „ II	Cornified
F 4	8	+ +	1 Type I 1 „ II	Cornified
F 5	8	+ +	2 Type I 3 „ II	Cornified
F 6	11	+ + +	13 Type I 10 „ II	Cornified
F 7	8	+ +	2 Type II	Cornified
F 8	8	+ +	3 Type I 5 „ II	6—7 layered mucified
F 9	12	+ +	None	Thick stratified
F 10	12	0	None	No reaction
F 11	8	0	None	No reaction
F 12	8	0	None	No reaction
F 13	8	0	1 Type II	No reaction
F 14	8	0	1 Type II	No reaction
F 15	8	0	1 Type II	No reaction
F 16	8	+	1 Type II 1 „ V	Cornified
F 17	12	+ +	1 Type II 1 „ V	Cornified

tained only small amounts of interstitial cells. If such grafts produced a response of the vaginal indicator, it would definitely exclude the possibility that the absence of reaction of the vaginal indicators in transplants B 1—8 was due to the number of interstitial cells being too small.

This series consisted of 17 transplants. All had a fair amount of morphologically normal corpus luteum tissue.

On dissection of very small aggregates of interstitial cells a certain degree of traumatisation is unavoidable. This explains why a relatively large number of transplants (F 10—15 in Table F) showed no survival of the interstitial cells. In those cases the vaginal indicator showed no reaction. Two transplants (F 16 and 17) were contaminated by follicles with theca interna, and in both the vaginal indicator was cornified.

Nine transplants contained surviving interstitial cells not contaminated with follicles possessing theca interna. In these cases the interstitial cells were fewer, often appreciably fewer, than in transplants B 1—8, and with but one exception (F 6) the number was approximately of the same order as that of the theca interna cells in those transplants of follicle walls (Chapter VII) that showed a good survival of these cells. Six (F 2—7) of these 9 transplants induced a pronounced reaction of the vaginal indicator, which responded with formation of thick cornified epithelium. One transplant (F 1) contained only a very small number of interstitial cells, and in that case, as in transplants E 1—4 the vaginal indicator showed only slight epithelial proliferation. Transplant F 8 showed a good survival of interstitial cells but only moderate epithelial reaction. In that case, however, the ovarian parts were lodged in the wall of the vaginal cyst, *i.e.,* in a less favourable position for acting upon the vaginal indicator. In transplant F 9, which also showed a good survival of the interstitial cells, both ovarian parts had fused with the vaginal graft, but the ovarian fragments were some distance apart from one another. The vaginal graft showed only slight epithelial proliferation. This suggests that these two parts must be in contact if they are to produce a more pronounced response of the vaginal indicator.

The vagina and uterus *in situ* and the control vaginal transplant showed castration atrophy.

Chapter X

Action of Follicles in the Transplants

It is apparent from the preceding sections that many of the transplants in the various series contained a varying number of different sized follicles as contaminants. Some of these follicles were of normal morphologic appearance, while others showed more or less pronounced degenerative changes. It could not be decided whether these changes were due to the transplantation *per se* or to normal follicle atresia. Neither was any attempt made to analyse the changes in further detail for the simple reason that it was not necessary for interpretation of the results of the experiments. Such follicles were therefore not classified separately in the tables but taken together with follicles showing no such changes. In the short-term experiments the larger follicles rarely showed degenerative changes. These follicles had presumably been so small at the time of transplantation that they had escaped detection, but had developed into large follicles during the experimental period.

The present investigation did not include transplantation of dissected whole follicles. For reasons given below, the test object in such a series should preferably have been follicles of Types III and IV *i.e.*, small follicles in different stages of development of the theca interna. Since such a series would have surely been valuable, attempts were made to dissect intact, uncontaminated whole follicles, but without success.

Analysis of the action of follicles of Types III and IV was therefore based on transplants containing only one or a few follicles of these types together with structures known not to be able by themselves to produce any reaction of the vaginal indicator. The number of such experiments described in the foregoing chapters was not sufficient for the purpose in view.

The material was therefore extended by addition of animals from parallel experiments not accounted for in the present study. These supplementary groups did not differ from the others in any relevant respect, *i.e.* they contained only 1 follicle (when of Type III, 1 to 3 follicles) combined with ovarian tissue unable of itself to produce any response of the vaginal indicator. Concerning follicles of Type V, a relatively satisfactory material was obtained without it being necessary to transplant whole uncontaminated follicles (see below). Moreover, since the control experiments had shown that it is extremely difficult to free such follicles from all extra-follicular tissue — small aggregates of interstitial gland cells and small follicles of Types I and II could always be demonstrated in serial sections — no attempt was made to increase this material still more.

Follicles with antrum (Type V). As shown (Chapter VII) even a small fragment of the wall of a large follicle of this type is sufficient to exert an oestrogen-like effect on the contiguous vaginal transplant. In the series in which attempts had been made to secure uncontaminated theca interna cells by scraping off the granulosa layer with a glass needle (Chapter V) there were 4 transplants (experimental period 16—25 days), each of which consisted of a Type V follicle (Fig. 43 and 44). Two of them also contained a few primordial follicles. The Type V follicles varied in size from 260 μ to 390 μ and they all had a well developed thecal gland. Since the scraped cupolas of the follicles succumbed, these follicles may be regarded as representing transplants of Type V follicles. In all of these cases the vaginal indicator showed massive cornification (Fig. 43) while the vagina and uterus *in situ* as well as the control vaginal transplant in the other eye showed no response at all.

In the series described in the previous chapters, there were 3 grafts, namely B 12 (in Table B and Fig. 22), E 14 (in Table E) and 1 of the 4 contaminated transplants of corpus luteum tops (see page 22 and Fig. 45 and 46) each containing a small follicle (230 μ to 310 μ) with initial formation of antrum. In these 3 cases the vaginal indicator was pronouncedly cornified, a reaction that could not be produced by such transplants without follicles. In other series there were 8 transplants (experimental

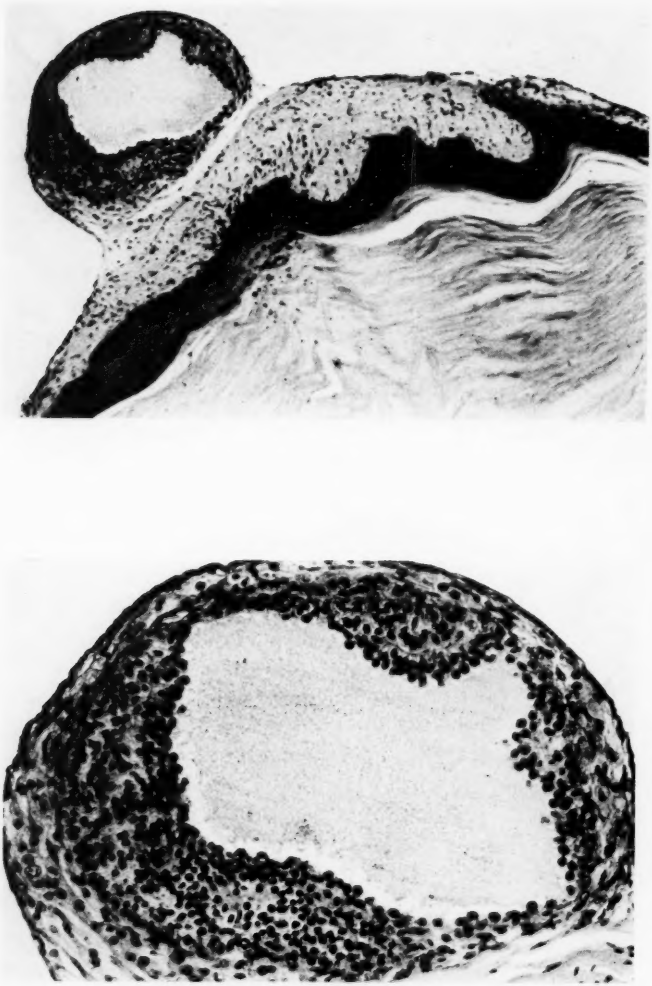
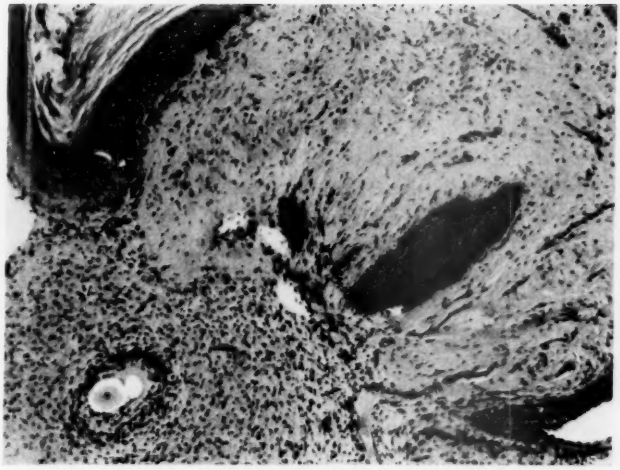


Fig. 43 and 44. Transplant containing only one medium-sized Type V follicle. Experimental period 24 days. The vaginal indicator shows continuous cornification. ×80 and 200.



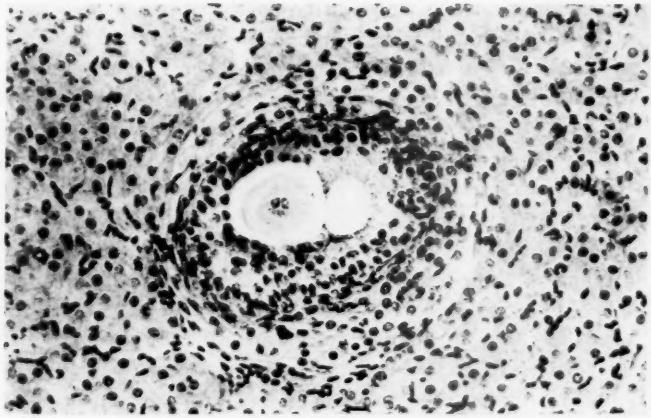


Fig. 45 and 46. Transplant of a corpus luteum top containing one small Type V follicle in initial formation of antrum. Vaginal indicator heavily cornified. ×80 and 200.

period 8—20 days) that contained only 1 Type V follicle. In those the contiguous vaginal grafts had likewise undergone marked cornification. In 5 of these cases, in 3 of which no other endocrine cell systems could be detected, the follicle was of preovulatory size with a well developed thecal gland. In these 5 experiments the vagina and the uterus *in situ* had reacted with manifestations of oestrus. Similar observations were made in transplant B 13 (in Table B) containing 2 Type V follicles, 1 of which was of preovulatory size. B 13 was the only transplant in the experiments accounted for in Chapters III—IX that produced a systemic response.

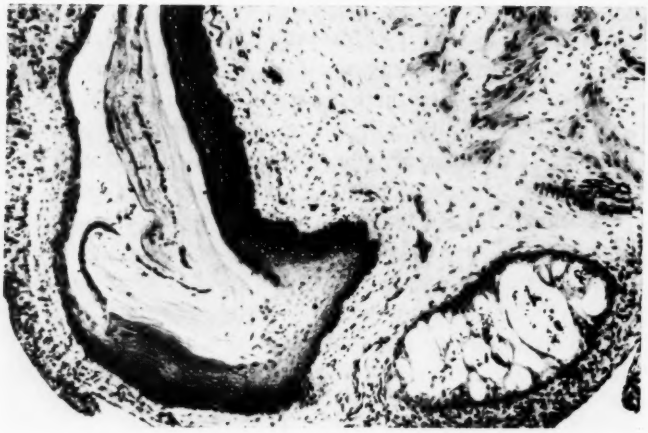
Small follicles with a well developed theca interna gland but without antrum (Type IV). As mentioned, attempts to transplant isolated uncontaminated follicles of Type III or IV proved unsuccessful. The series described included 2 transplants, A 11 (Fig. 47 and 48) and B 15 and, the other series, 2 (experimental period 15 and 20 days) each containing 1 follicle of Type IV, but no other structures capable of themselves of inducing any reaction of the contiguous vaginal epithelium. In the series of scraped follicle cupolas combined with granulosa cells 1 transplant contained one Type IV follicle and luteinised granulosa cells. In these cases the vaginal indicator had responded with cornification. A sixth transplant (B 11) of essentially the same type and containing 2 follicles of Type IV and a few smaller follicles showed, however, only a weak reaction. Possible explanations for such unexpected negative results are suggested in Chapter XI.

Small follicles with few theca interna gland cells (Type III). The material summarised in the tables included 6 transplants (A 14, 16, 18 and 20 and B 9 and 14) containing 1—3 follicles of this type. These follicles thus had adherent ovarian structures unable of themselves to produce any response of the vaginal epithelium. In transplants A 14 and 16, each of which contained one follicle, the contiguous vaginal transplant showed a slight to moderate proliferation of the epithelium, while transplants A 18 and 20, which likewise contained a follicle of this type, produced no reaction of the vaginal epithelium at all. On the other hand, transplants B 9 and 14, which were contami-

nated with 2 respectively 3 follicles of this type, produced a marked response with the formation of thick squamous epithelium, which in B 14 was cornified. Two of the 4 above mentioned (page 22) contaminated corpus luteum transplants contained a follicle of this type but no other follicular structures. In these cases the vaginal indicator epithelium responded in the same way as in transplants A 14 and 16. In other series there were 7 transplants (experimental period 8—15 days) each containing 1 or 2 Type III follicles but no other structures capable by themselves of inducing any response of the vaginal epithelium. In 2 of these experiments the vaginal indicator showed no reaction, while in the remainder the indicator responded with epithelial proliferation and in 2 cases (in which the transplant contained 2 follicles) with cornification as well.

In the series in which the follicle cupolas had been scraped clean with a glass needle (Chapter V) 1 of the transplants (experimental period 24 days) contained a follicle of Type III (Fig. 49 and 50) as well as a relatively large follicle of Type II in degeneration (see Fig. 50), and a very small follicle of Type II. The theca interna cells of the scraped cupola had succumbed. Within a small region close to the ovarian part of the graft the vaginal indicator showed thick, squamous and partly cornified epithelium in spite of the fact that the follicle was in the wall of the vaginal cyst, *i.e.*, in a less favourable position for action on the indicator.

Primordial follicles and small follicles without theca interna gland cells (Types I and II). In the series of scraped follicle cupolas (Chapter V), 5 of the transplants that had been in the eye for 8—24 days were found to contain a varying number of these types of follicles, but no other ovarian structures since the theca interna cells in the transplanted cupolas had succumbed. One of the transplants contained 10 follicles of these types. In none of these 5 grafts did the contiguous vaginal epithelium show any response. It is clear from Chapter III and Tables A and B that follicles of Types I and II together with theca interna cells, interstitial cells or corpus luteum cells had no effect on the vaginal indicator, not even when many such follicles were present in the grafts (see B 6 in Table B). Cor-



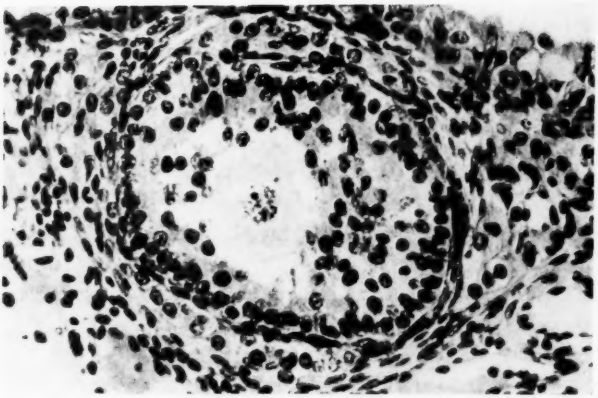
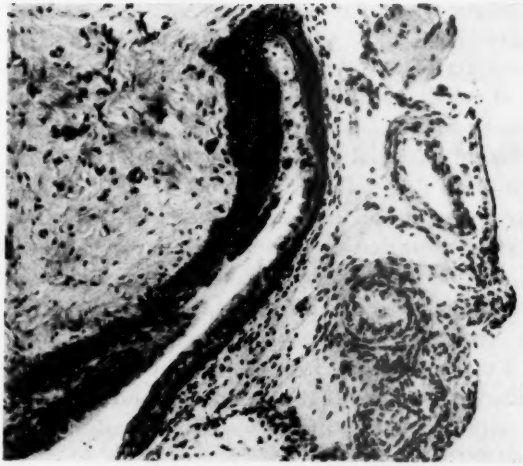


Fig. 47 and 48. Transplant (A 11) of theca interna cells contaminated with one Type IV follicle (only seen in Fig. 48). Vaginal indicator with thick cornified epithelium. ×90 and 310.



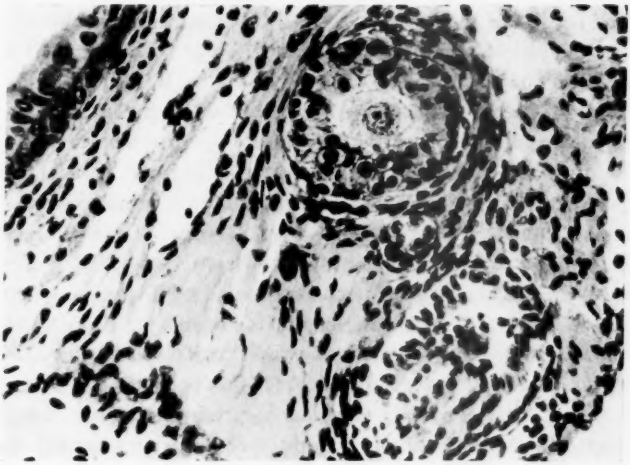


Fig. 49 and 50. Ovarian graft containing one Type III and two Type II follicles. The vaginal indicator has responded with formation of thick, slightly cornified epithelium. ×125 and 310.

responding results were obtained with essentially similar transplants (6 cases) in other series. The material clearly shows that small numbers of follicles of Types I and II in the ovarian grafts had no demonstrable effect on the epithelium of the vaginal indicator.

The present material thus provided no direct evidence for the assumption that follicles of Types III and IV *per se* can induce any reaction of the vaginal indicator. The experiments did, however, show that these types together with ovarian structures unable of themselves to induce any reaction of the vaginal epithelium had a clear effect. Type IV produced the strongest effect with cornification of the contiguous vaginal epithelium in 5 of the 6 cases. Type III, on the other hand, had an effect ranging between that of Type IV and that of Type II. The strength of reaction of the vaginal indicator did not seem to vary with the amount or type of contiguous endocrine tissue (interstitial gland, theca interna cells or corpus luteum cells) but only with the type of follicle.

The results of the experiments with ovarian grafts containing various types of follicles are summarised in Table G. It is evident that one large or medium-sized Type V follicle alone is capable of inducing a heavy cornification of the vaginal indicator. A large follicle of this type may even produce an oestrous reaction in the vagina *in situ* and uterus. It is also evident that grafts containing only one of the ovarian endocrine cell systems (not of themselves producing any oestrogenic effect on the vaginal indicator) in combination with one small Type V follicle with initial antrum formation or one of Type IV or 1 to 3 of Type III produce an oestrogenic (or oestrogen-like) reaction in decreasing order of strength, while grafts containing several follicles of only Types I and II are inactive. Much argues for the assumption that this reaction is produced by the follicles *per se*. Theca interna and granulosa cells in large or medium-sized Type V follicles in combination give an oestrogenic (or oestrogen-like) effect and the indicator response of the grafts with the smaller follicles is well correlated with the degree of development of the thecal gland. On the other hand, there was no obvious correlation with the other cell systems present in the

TABLE G

Action of the various types of follicles in ovarian grafts

+ + +: marked cornification; + +: moderate cornification; +: slight cornification; 0: no response.

Types of follicles in the ovarian graft	Other structures in the ovarian graft	Response of vaginal indicator
Large Type V (one follicle or one piece of follicle wall)	0	+ + +
Medium-sized Type V (one follicle)	0	+ + +
Small Type V (one follicle)	Interstitial cells or corpus luteum cells	+ + +
Type IV (one follicle)	Interstitial cells, theca interna cells from large Type V or corpus luteum cells	+ to + +
Type III (one to three follicles)	Interstitial cells, corpus luteum cells or theca interna cells from large Type V	0 to + +
Types I and II (several)	Interstitial cells, corpus luteum cells or theca interna cells from large Type V	0

ovarian transplants and thus no reason to assume the necessity of any interaction between the small follicles and these cell systems for the production of the effect. This is strengthened by the finding that in one graft (see above under Type III follicles) containing only one Type III and two Type II follicles, but no other observable endocrine cells, the epithelium of the vaginal indicator was cornified.

It thus seems that the ability of follicles to produce an oestrogenic (or oestrogen-like) effect is dependent on the presence of theca interna gland cells and that the degree of this ability is correlated with the degree of development of this gland. It might also be correlated with the degree of maturity of the granulosa cells, but the experiments do not permit any valid conclusion on this point. An observation suggesting that the

degree of maturity might be of relevant significance is that in transplants of the type found in B 6 (Table B), which contained several to many Type II follicles, the granulosa cells did not interact with the interstitial cells in the grafts to produce any effect on the vaginal indicator, while on the other hand a marked effect was sometimes produced by a single Type III follicle.

Other effects of follicles Type V. In transplants containing follicles of this type the contiguous vaginal epithelium always showed heavy cornification with thick layers of horny lamellae. But in some experiments this reaction was most advanced in regions of the epithelium farthest from the ovarian part of the graft, while the epithelium closest to the ovarian tissue showed only slight or no cornification. In those cases in which the epithelium closest to the ovarian part of the graft showed no signs of cornification, this epithelium was of varying appearance (thick squamous epithelium, or thick epithelium with voluminous surface cells, sometimes of the mucin cell type). Thus, there appeared to exist some factor inhibiting cornification.

In other series in which the survival and behaviour of microtransplants was studied in long-term experiments some of the transplants contained follicles of Type V undergoing transformation into corpora lutea (most frequently with retained egg cell). In those cases cornification of the vaginal indicator was more or less completely inhibited: the epithelium was thick and contained voluminous surface cells often of mucin type while the vagina *in situ* showed typical oestrous reaction. In the transplants in which the follicles had been transformed completely into corpora lutea the same phenomenon was observed in spite of the fact that they were surrounded by abundant theca interna cells. No such effect was ever seen in experiments using transplants made up of granulosa or corpus luteum cells combined with interstitial cells or theca interna cells (Chapters VII—IX).

It has long been known that the cornifying effect of oestrogens can be blocked by certain steroids including progesterone (*cf.* COURRIER 1950) when given in sufficient amounts. There is a growing body of evidence (*cf.* FORBES 1953, EDGAR 1953,

ZANDER 1954) that progesterone can be secreted in the preovulatory phase in the absence of corpora lutea. It is therefore tempting to assume the existence of a progesterone effect in these transplants. Investigations are in progress (with another type of indicator system) to determine whether and, if so when, follicles of different types form steroids with a progesterone effect.

Chapter XI

Discussion of the Experiments

RESPONSE OF THE VAGINAL INDICATOR

It is well known that in certain rodents, *e.g.* the rat, gonadotrophically stimulated ovarian transplants are capable of inducing stratification and cornification of the vaginal epithelium *in situ*. This vaginal response has been accepted as a sign of production of oestrogenic hormone by the grafts. Much evidence has accumulated in support of this interpretation and no experimental findings arguing against it seem to exist. In the discussion of experiments, such as those described in the present paper, designed to elucidate the site of production of oestrogen in the ovary, the specificity of the response of the vaginal indicator needs further discussion.

1. *Is the response of the vaginal indicator unspecific in the sense that it is not induced by steroids produced in the ovarian graft?*

Evidence against the possibility of the response not being specific was presented in Chapter II. It might be mentioned that in the preliminary, present, or parallel experiments with vaginal transplants with or without a contiguous ovarian graft in a total of more than 700 animals no findings were made that argued against the response of the vaginal graft being specific. It seems highly improbable that the various ovarian components, *e.g.* the theca layer of follicles, interstitial tissue, corpus luteum tissue or granulosa cells, which did not of themselves produce any response of the indicator, should produce an unspecific oestrogen-like reaction when present in certain combinations in the grafts. Furthermore, it is well known that vaginal auto-transplants do not react with an oestrogen-like response to entirely different non-steroid-producing host tissues.

This also argues strongly against the possibility of the ovarian tissue containing any factor capable of inducing an unspecific response.

2. *Is the response of the vaginal indicator due to a local action of steroids primarily formed by the ovarian graft?*

Since neither the control vaginal graft in the other eye nor the vagina *in situ* showed any response, it appears that the steroids released did not first enter the general circulation and then — possibly after being metabolised to steroids with oestrogenic properties — reach the vaginal indicator and, secondly, that the response of the indicator was not due to any substances with oestrogen-like effects from elsewhere, *e.g.*, the adrenal cortex. The fact that the response of the vaginal indicator was most pronounced in the part closest to the ovarian graft also shows that the action was a local one.

It thus seems reasonable to assume that the response of the indicator is induced by the local action of steroids released from the ovarian graft.

3. *Is the response of the indicator produced by the oestrogenic hormone(s) of the rat ovary or by some other steroid(s)?*

It is known that under certain circumstances some steroids of the androstane and pregnane series are capable of inducing stratification and even cornification of the rat vaginal epithelium. It has been claimed (see Chapter XII) that the ovary elaborates androgens and that the response of the indicator may therefore possibly be due to the release of androgens or progestins or some intermediary metabolite in the synthesis of these hormones.

A large number of steroids of the androstane and pregnane series have been studied for their ability to induce cornification of the rat vaginal epithelium (WARREN 1935, DEANESLY and PARKES 1936, 1937, NELSON and GALLAGHER 1936, KORENCHEVSKY *et al.* 1937, KORENCHEVSKY and HALL 1937, NELSON and MERCKEL 1937, NATHANSON *et al.* 1938, PARKES and ZUCKERMAN 1938, KORENCHEVSKY 1939, NOBLE 1930, MAZER and MAZER 1940, CLARKE and SELYE 1942, SELYE and CLARKE 1944, HUG-

GINS and JENSEN 1954, HUGGINS *et al.* 1954). Even if the results of these studies are conflicting in some respects, they do show that some of these steroids have a cornifying effect on the vaginal epithelium in the immature normal and spayed rat and in the adult normal rat, but only when administered in large doses. But with the exception of Δ^5-androstene-3β,17β-diol, these steroids have no such effect on the vaginal epithelium in the adult spayed rat, not even when given in large doses. It thus seems reasonable to assume that in the normal adult rat this oestrogenic effect is exerted either after transformation of the steroids in the body or by stimulation of the ovaries to production of oestrogen. It has been shown that androgens can be converted to oestrogens in the animal body as well as in ovarian tissue *in vitro* (for references see DORFMAN 1957 and HECHTER 1958). No satisfactory explanation for the exceptional behaviour of the steroids in the *immature* rat is as yet available. This phenomenon, however, is irrelevant in the present discussion. It has repeatedly been confirmed that Δ^5-androstenediol is a unique compound in the C_{19}-series in that it can induce cornification of the vagina in the spayed adult rat. It is true that it requires a relatively enormous dosage compared with natural oestrogens, but this does not fully exclude the possibility of this compound having a weak oestrogenic activity *per se*. EMMENS (1941), however, found that the median effective (cornifying) dose of Δ^5-androstenediol was the same whether applied intravaginally or subcutaneously. As pointed out by EMMENS (1941), this strongly suggests that the oestrogenic activity of this compound is due to its conversion to oestrogenic metabolites.

None of the numerous investigated biogenic steroids not belonging to the series of natural oestrogens has been demonstrated to possess the property *per se* to induce cornification of the rat vaginal epithelium. It thus seems unlikely that steroids of the androstane and pregnane series — even if released from the ovarian grafts — should have been able to produce cornification of the vaginal indicator *by a local action*. This possibility cannot, of course, be definitely ruled out.

It might be argued that the ovarian grafts do not produce oestrogen(s) but some unknown substance capable of exerting

oestrogen-like effects upon the vaginal indicator. There seems to be no reason, however, to complicate the interpretation of the results in the present paper by introducing purely hypothetical objections of such general character. On the one hand, it has long been known that steroid-producing cell systems (adrenal cortex, testis, ovary) — unless exposed to abnormal influences — exert essentially the same hormonal effects whether they are situated in grafts or *in situ*. In other words, the steroid production in the transplanted cells does not differ fundamentally from that of the cells *in situ*. On the other hand, it was shown in the experiments described in the previous chapters that certain of the transplanted ovarian cells produced an indicator response of the oestrogen type. This, together with the general considerations set forth above on the indicator response, motivates the conclusion that when the vaginal indicator responds with stratification and cornification of its epithelium, this response is due to release of oestrogen from the ovarian cells.

In the following discussion an indicator response of this type will be taken as a sign of a release of oestrogen from the ovarian graft. Though this assumption appears to be well founded, it must be borne in mind that even if the epithelial response to oestrogen is highly specific, it is not known with certainty whether the epithelium might not be capable of responding in the same way to some other substance.

SITE OF OESTROGEN PRODUCTION — VALIDITY OF RESULTS

When transplanted by itself no endocrine cell system of the rat ovary exerted any demonstrable oestrogenic effect on the vaginal indicator. On the other hand, transplants containing theca interna cells or interstitial gland cells *combined with* granulosa cells or corpus luteum cells proved to possess the ability to produce oestrogen. It thus seems as if oestrogen formation is dependent on some sort of joint action of two different cell systems.

The results are not conclusive, however, until it has been demonstrated that the experimental method used did not in

some way prevent the grafts of "pure" cell systems from exerting a possibly existing oestrogenic function. Conceivable experimental factors responsible for the lack of activity might be functional damage of the transplanted cells, the use of too small a number of cells to evoke a reaction of the vaginal indicator or exposure of the cells to inadequate gonadotrophic stimulus.

As already emphasised concerning the theca interna cells (see Chapters V and VIII), it does not seem probable that the cells were damaged more severely when transplanted alone than when transplanted in combined grafts. The technique applied for the transplantation of a given cell system was always the same whether the cells were transplanted alone or combined, so that a systematic technical difference allowing a given cell system to produce oestrogen only in the series of combined grafts seems inconceivable. Furthermore, it was shown that the interstitial cells in the transplants of corpus luteum bases and in the combined transplants with corpus luteum cells were definitely more severely traumatised (see Chapter IX) than the cells transplanted alone.

The possibility that the quantity of cells might have been too small has already been ruled out concerning transplants of theca interna cells (see Chapter VIII) and of interstitial cells (see Chapter IX). As to the transplants of granulosa cells and corpus luteum cells, they were generally the largest transplants, particularly the transplants of corpus luteum tops, of which 2—5 were used for each graft.

It seems highly improbable that the gonadotrophic stimulus should have been inadequate for the isolated cell systems, since it stimulated the combined transplants and the larger types of follicles to produce oestrogen.

Other arguments for the validity of the results can also be put forward. It may be stressed that the grafts of "pure" cells *in no case* exerted any oestrogenic effect, which the combined and unintentionally contaminated grafts generally did. It is well known that the theca interna and interstitial gland cells are closely related, as are the granulosa and corpus luteum cells, and all the experimental series showed that oestrogen is formed

in a graft consisting of either cell type of the first system combined with either cell type of the second. The analysis of the action of follicles in the grafts yielded further evidence in that it showed that the ability of follicles to secrete oestrogen probably arises with the development of the theca gland cells. This systematic nature of the results speaks in itself for their validity.

As exemplified in the previous chapters, in some cases the response of the vaginal indicator to the ovarian graft was not so strong as expected. In this type of experiment there are obviously many variables capable of interfering with the response, such as inter-individual biological variations in the sensitivity of the vaginal epithelium or in the oestrogen-producing capacity of the corresponding cell systems. Further, the possibilities of the oestrogen released from the grafts to reach the vaginal epithelium may vary. Such unexpectedly weak responses, however, do not limit the validity of the results.

The validity of the experimental results therefore appears to be good and there seems to be no reason to suppose that the demonstrated joint action of two cell systems in the oestrogen formation is simply due to some transplantation artefact. *It may be concluded that the experiments strongly support the view that none of the four different endocrine cell systems of the rat ovary is of itself capable of producing oestrogen, but that the production of hormone depends on a joint action of the two principal ovarian systems: the system of theca interna and interstitial gland cells on the one hand and the system of granulosa and corpus luteum cells on the other hand.*

POSSIBLE INFLUENCE OF THECA INTERNA CELLS IN THE CORPUS LUTEUM TRANSPLANTS

The fate of the theca interna cells in the development of the corpus luteum in the rat ovary still remains obscure. While some authors claim that the theca interna gland cells actually take part in the formation of corpus luteum and transform into lutein cells, others are of the opinion that they disappear in an early stage in the development of the corpus luteum (for references see HARRISON 1948, PEDERSON 1951 and BRAMBELL 1956).

The results of the transplantation experiments with corpus luteum tops showed that theca interna gland cells, if present in the corpus luteum, did not produce any — or at least not enough — oestrogen to give an indicator response. It might be argued that a possibly occurring oestrogenic effect might have been inhibited by progestin released simultaneously from the corpus luteum cells. This seems unlikely, however, since — as is well known — progestin produced by the corpora lutea of the rat ovary interacts with oestrogen and the combined action of these hormones renders the vaginal epithelium thick and mucified, and no such response of the indicator was observed. It seems most probable that after ovulation the theca interna cells either degenerate or develop into cells without any direct oestrogenic function. This is in good agreement with the results obtained in studies of the histochemistry of rat corpora lutea (DEMPSEY and BASSETT 1943, EVERETT 1945, CLAESSON and HILLARP 1948, DEANE 1952 and DAWSON and VELARDO 1955), which show that the theca lutein cells — if such cells do really exist — do not possess the typical capacity of theca interna and interstitial cells to store large amounts of cholesterol during certain of the reproductive phases.

Chapter XII

General Discussion

It is generally accepted that the corpus luteum is the source of ovarian progesterone and there is good evidence that this hormone can be produced already by the granulosa cells (FORBES 1953, EDGAR 1953, ZANDER 1954, GREEN 1955, 1956). But there is still divergence of opinion about the site of production of oestrogen.

In the first important investigations, especially those by ALLEN and his collaborators and by ZONDEK and ASCHHEIM, different ovarian structures were studied for the presence of oestrogen. ALLEN *et al.* (for references see ALLEN 1941) demonstrated that follicular fluid, follicle wall and human corpus luteum contain oestrogen, and they stated that the follicular epithelium was the primary source of oestrogen. ZONDEK and ASCHHEIM (for references see ZONDEK 1931), however, showed that after removal of the granulosa layer the follicle wall also contained oestrogen, but they failed to demonstrate any oestrogenic activity in the granulosa cells. From these and other findings they concluded that the theca interna and the histogenetically closely related interstitial gland was the site of production. ZONDEK (1931) also believed that the granulosa cells and corpus luteum cells in the human ovary secreted not only progesterone but probably also oestrogen.

An observation favouring the view that theca interna is oestrogen-producing was reported by MOSSMAN (1937), who found that in *Geomys bursarius* the thecal gland reaches an enormous development coinciding with high oestrogenic activity of the ovary. In an analysis of the literature dealing with this problem CORNER (1938) concluded that "the most probable site of production in the ovary is the theca interna of follicles of

all sizes". In their investigation of the guinea-pig ovary STAFFORD *et al.* (1942) pointed out that the theca interna in that animal is best developed at the time of oestrus, which they found was in agreement with similar findings in other animals. They concluded that the theca interna is probably the source of oestrogen. Several other attempts have been made to correlate ovarian hormone formation with the cytology of the ovarian cells, but with contradictory results. Some investigators (see *e.g.* DEANE and BARKER 1952) found theca interna, others (see *e.g.* FREED and SOSKIN 1937, HERNANDEZ 1943) both the theca interna and granulosa or the granulosa alone to be the site of oestrogen formation. The difficulties in the interpretation of the results obtained in studies of this type are obvious. They may be exemplified further by the work of GREEP (1938), in which it was concluded that "the interstitial cells may be eliminated from consideration as the source of oestrogen" in ovaries of hypophysectomised rats treated with human chorionic gonadotrophin. He then assumed the persisting corpora lutea to be the oestrogen-producing structure but now holds the view that it is the theca interna that is most probably concerned (GREEP and JONES 1950).

It seems to be generally accepted that the interstitial gland of the rat ovary is formed at least mainly by theca interna cells from atretic follicles, though the interstitial gland in the very young rat seems to be of another origin (DAWSON and MCCABE 1951). The close relationship between these two glandular systems is further suggested by their similar histochemical and cytological appearance and reactions to gonadotrophins. After hypophysectomy these cells rapidly transform into the deficiency-cell type (SELYE, COLLIP and THOMSON 1933) but respond in the same manner and specifically on stimulation with interstitial cell stimulating hormone (ICSH) or human chorionic gonadotrophin (HCG) by again assuming the appearance of typical gland cells. CLAESSON *et al.* (CLAESSON and HILLARP 1947, CLAESSON *et al.* 1948, 1953, ALDMAN *et al.* 1949, CLAESSON 1954) made an extensive investigation on the lipids, mainly the cholesterol fraction, in the theca interna and interstitial gland. They demonstrated a gonadotrophically regulated variation of the

cholesterol content of these cells. The correlation between the variation and the secretion of oestrogen was such that it seemed probable that the theca interna and the interstitial gland represented the oestrogen synthesising cell system working with cholesterol as a precursor.

Their findings are in agreement with those of RENNELS (1951) who studied the histochemistry of the interstitial gland of the immature rat ovary and of FALCK (1953), who demonstrated that the transition of the infantile rat ovary from a non-oestrogen producing to an oestrogen-producing stage coincides with a commencing storage of cholesterol in the interstitial gland.

The significance of these histochemical studies has recently been augmented by the production of direct evidence that cholesterol is a precursor of oestrogen (for references see HECHTER 1958).

The possibility that the interstitial gland and theca interna gland together constitute the oestrogen-producing cell system of the ovary is supported by the well-known observation — first made by SELYE, COLLIP and THOMSON (1933) — that HCG when administered to adult hypophysectomised rats induces repair of the deficiency cells together with formation of oestrogen without exerting any demonstrable effect on the atrophied follicular apparatus.

The results obtained in studies on oestrogen formation in ovaries after x-ray irradiation are difficult to interprete and partly contradictory (*cf.* PARKES 1926, 1928, BRAMBELL and PARKES 1927, WESTMAN 1930, GELLER 1930, GENTHER 1931, 1934, PARKES, ROWLANDS and BRAMBELL 1932, GENTHER SCHMIDT 1936, 1937, 1939, MANDEL 1934—35, GEIST *et al.* 1941). Although the experiments clearly show that an intact follicular apparatus is not necessary for the formation of oestrogen, they do not clearly indicate which of the cells persisting after irradiation are the source of oestrogen. Furthermore, the experiments are inconclusive as regards the origin of the persisting cells (*cf.* CORNER 1938).

Although the investigations point to the theca interna and interstitial gland as the main site of oestrogen formation in the ovary, the evidence obtained is indirect and thus allows other

interpretations. This is perhaps best illustrated by the statement of ALLEN (1941): "I think there is now evidence that all ovarian tissues may secrete oestrogen — granulosa, theca, interstitial and luteal — but that follicular epithelium is probably normally the primary source."

The problem of the ovarian hormone formation has been complicated further by the claims in a large number of studies that the ovary secretes not only oestrogen and progestin, but also androgenic hormone. The most pertinent experiments as well as references to other studies are found in the papers of WESTMAN and JACOBSOHN (1944), HILL (1937, 1941), DEANESLY (1938), GREENE and BURRIL (1939), MARX and BRADBURY (1940), BURRIL and GREENE (1941), PFEIFFER and HOOKER (1942), HERNANDEZ (1943), PRICE (1944, 1947), KATSH (1950), PARKES (1950), RENNELS (1951) and KULLANDER (1956). There seems to be good evidence that the rat ovary under certain — usually rather abnormal — conditions (immaturity, long-term grafting, excessive gonadotrophic stimulation or stimulation with gonadotrophins not of pituitary origin) may produce substances with androgenic activity, but so far there is no conclusive evidence that the normal ovary of the adult rat actually has any significant androgenic function. Although in these studies "luteinised" theca interna or interstitial cells have generally been supposed to be the source of the androgens released, the evidence for this view is meagre and of very indirect nature. Since it is now known that the synthesis of oestrogen probably involves C_{19}-steroids as intermediary metabolites, it seems quite possible that the experimental findings may be explained without postulating the presence of ovarian cells specialised for androgen secretion. The androgens probably secreted under certain conditions may well be metabolites released from the oestrogen-producing cells owing to a changed metabolism under the influence of an inadequate or excessive gonadotrophic stimulus or other "abnormal" factors resulting, for example, in a more or less complete block of the final steps in the oestrogen synthesis. This would thus be a phenomenon analogous with the release of steroids from the adrenal cortex under certain abnormal or hyperactive conditions, which normally function as intermediaries in the

corticoid biosynthesis (*cf.* HECHTER 1958, SAFFRAN and SAFFRAN 1959).

In view of the accumulated evidence pointing towards the theca interna and interstitial cells as the source of ovarian oestrogen, it was at first surprising to find that these cells did not exert any oestrogenic activity when grafted alone. This contradiction was only apparent, however, and was explained by the subsequent finding that the oestrogen production seemed to depend on a joint action of the two principal ovarian cell systems. Since there were previously no obvious reasons — with one exception (see below) — to assume that the different types of secretory cells in the ovary might not be able to operate independently of each other in their production of hormone, the various experimental findings in the literature were interpreted on the basis of this postulate. In fact, the experimental method used in the present study was originally designed on the basis of this supposition. The conflicting interpretations of experiments performed during the last three decades to elucidate the source of ovarian oestrogen may now find a reasonable explanation, *i.e.*, they were based on a premise, the validity of which now must be seriously questioned.

The findings in the present study indicate that the oestrogen production in the rat ovary is dependent on some sort of joint action of the system of theca interna — interstitial cells on the one hand and the system of granulosa — corpus luteum cells on the other hand. The nature of such interplay is, however, still obscure so that it cannot be decided by which of the cells the oestrogen is actually secreted.

It is well established — since the basic investigations of DEMPSEY and BASSETT (1943) and CLAESSON and HILLARP (1947) — that the theca interna cells possess the characteristic features of steroid-producing cells and thus probably secrete steroids. Several investigations approaching the problem from different angles and referred to above have secured further indirect evidence that the theca interna cells are probably one of the sources of ovarian oestrogen. The experiments in the present study yielded direct evidence of their being involved in the formation of oestrogen. Taken together these facts weigh heavily for the view

that the theca interna cells actually secrete oestrogenic hormone. Other considerations may be brought forward in support of this view. Transplants containing theca interna in combination with either granulosa, luteinised granulosa or corpus luteum cells were shown to produce oestrogen. If the hormone is not secreted by the theca interna it must emanate from the other types of cells. Since it is well established that the luteinised granulosa cells secrete the ovarian progestin, they should then secrete two types of hormone. This seems less probable, however, since extensive research in ovarian physiology has failed to demonstrate any such dual steroid secretion.

Despite the close relationship between the interstitial gland cells and the theca interna cells, interstitial cells have less often been regarded as a source of oestrogen. Strong arguments for their ability to produce oestrogen have been brought forth by CLAESSON *et al.* (summarised in the papers of CLAESSON 1954). The transplantation experiments in the present study have shown, that the interstitial cells — like the theca interna cells — are capable of oestrogen formation in joint action with corpus luteum cells. These cell types are thus closely related also functionally. For the same reasons as those given above for theca interna it must be assumed that the interstitial cells are able to secrete oestrogenic hormone. In view of some peculiar features of the interstitial gland — such as the unexplained variations in its occurrence and in its development during the reproductive phases in some mammals — it seems possible, however, that this gland may also have other functions.

If the oestrogenic hormone really is secreted by the theca interna and interstitial cells, it must be assumed that the granulosa — corpus luteum cells play a mainly indirect role in the abovementioned joint-action mechanism. It seems most reasonable to assume that the former cells are in some way dependent on the latter. Before further analysis of this problem it might be convenient first to consider the possibilities of existence of such intra-ovarian mechanisms.

Many observations suggest that oestrogen released in the ovary may have significant intra-ovarian effects. Since the first studies of WILLIAMS (1940, 1944, 1945) it has been repeatedly con-

firmed (PENCHARZ 1940, SIMPSON *et al.* 1941, ARON and ARON 1951—52, WIT 1953, PAYNE and HELLBAUM 1955, PAYNE and RUNSER 1958) that oestrogens administered to animals act directly on the ovarian follicles. The follicles are stimulated to increased growth by hyperplasia and proliferation of the granulosa cells, and this is even more striking when the oestrogens act in synergism with gonadotrophins. The granulosa seems to be the target cells for this oestrogenic action. Owing to their close proximity to the theca interna, locally released oestrogen may normally possibly act on the granulosa. There are reasons to believe that this is so (see BULLOUGH 1943 and HISAW 1947). Furthermore, it has been shown (WESTMAN and JACOBSOHN 1937, ROBSON 1937) that oestrogen administered to hypophysectomised, pseudo-pregnant rabbits maintains the morphological integrity as well as the secretory function of the corpus luteum cells. That this effect is produced by direct action of the oestrogen has been confirmed (HAMMOND and ROBSON 1951, HAMMOND 1952), and the experiments of WESTMAN (1934) suggest that oestrogen released in the ovary may be of importance for the function of the corpus luteum cells.

The demonstration of the direct action of oestrogen on the granulosa — corpus luteum cells suggests that these cells may be directly influenced by the oestrogen-producing ovarian cells. If so, it would be an interesting dependency relation, complementary and opposite to that found likely in the present study, and there would thus be two intra-ovarian mechanisms connecting the two principal cell systems, the oestrogen- and the progestin-producing, with each other. The existence of other intra-ovarian mechanisms has been suggested by MOSSMAN (1952). His interesting observations on the porcupine ovary seem to show that the corpus luteum cells may influence the theca interna and interstitial cells. The finding of EVERETT (1941) that repair of the interstitial cells — after progesterone-induced ovulation in persistent-oestrous rats — was most advanced in the gland lobes adjacent to the newly formed corpora lutea may also argue for such an influence, although EVERETT is inclined to believe that this phenomenon is linked to vascular dilatation.

The assumption that the oestrogen-producing cells are depen-

dent for their activity on the granulosa or corpus luteum cells raises the question as to what way the latter cells may influence the former. The most reasonable — and probably the only possible — explanation is a release of substances which after transportation through the ovarian tissue to the oestrogen-producing cells are able to maintain or stimulate some — still unknown — processes in these cells. In analogy with the indicated intra-ovarian action of released oestrogen, it seems possible that the substances released from the granulosa-corpus luteum cells may be the progestational hormone itself or some intermediary steroid metabolite.[1] The construction of the follicle offers ample opportunities for substances to pass between the theca interna and granulosa, and it is tempting to relate the peculiar arrangement of the cells in the follicle wall to the suitability this arrangement offers for the cells to influence each other (*cf.* HISAW 1947). It also seems to be easy for substances to be transported to the interstitial cells. The transport possibilities are obviously good via blood and/or lymphatic vessels and by direct diffusion. It has been shown that steroid hormones can, by a direct route, act over a fair distance on neighbouring target organs (SUZUKI *et al.* 1955, GITSCH 1958) and MOSSMAN (1952) has described the existence of intra-ovarian long-distance transportation of substances produced by the corpus luteum in the porcupine ovary.

The analysis of the action of follicles in the ovarian grafts made it probable that follicles of all sizes containing theca interna gland cells are able to secrete oestrogen. The experiments also showed that a single follicle of pre-ovulatory size produced this hormone in quantities large enough to induce systemic effects, both the vagina and uterus *in situ* showing an oestrous

[1] In unpublished experiments together with K. MENANDER and O. NORDANSTEDT the possibility was checked whether the theca interna cells are dependent on progesterone for their oestrogen production. Grafts of isolated theca cells were exposed to progesterone (crystals in the eye or systemic injections). In several cases the vaginal indicator responded with cornification or — more often — with formation of a thick mucified epithelium. The latter response — in the presence of progesterone — also indicates a release of oestrogen. The experimental series is, however, as yet not large enough for the results to be regarded as conclusive.

response. On the other hand, a single small follicle with few theca interna cells and without antrum could produce a clear oestrogenic response of the vaginal indicator. Since the theca interna cells must be regarded as the primary source of oestrogen, some quantitative data on their number in these types of follicles might be of interest. Determinations showed that 30—40 000 theca interna cells were sufficient to produce systemic effects, but only 50—100 were necessary to cause a local reaction of the indicator. These data and the finding that a single large follicle may produce a full oestrous reaction of the vagina and uterus give a rough idea of the high secretory capacity of the theca gland cells and illustrate the important role of the follicular apparatus in the formation of oestrogens.

The assumption that the follicles play a fundamental role in the secretion of ovarian oestrogens dates back to the early days of ovarian physiology, when it was discovered that an intimate correlation exists between the development of the follicular apparatus and the oestrogenic activity of the ovary. This correlation was in fact one of the reasons that led ALLEN to postulate the primacy of the follicular epithelium. Since it now seems necessary to assume that the theca interna is the oestrogen-producing system in the follicle wall but also that this system is dependent on the follicular epithelium for its secretory activity, the view put forward by ALLEN and the later assumption by many investigators that the theca interna is the primary source of oestrogen, may now be brought into line with one another. Thus, the follicle as an entity may once again be regarded as a fundamental functional unit in the ovarian hormone formation.

Unfortunately our knowledge of the actions of the various gonadotrophic hormones on the ovary and particularly of what cells these hormones primarily affect is far from complete. It is therefore difficult to find out how the concept of the follicle as a secretory unit fits the data obtained in this field of research. The known effects of highly purified gonadotrophins on the ovarian oestrogen secretion in hypophysectomised rats may, however, be taken as suggestive evidence for the view that the oestrogenic function of the theca interna cells is dependent on some activity of the follicular epithelium. It is well known that

the follicle stimulating hormone (FSH) and the ICSH produce follicle growth and repair of the deficiency-cells, respectively, as the only microscopically recognisable effects. Neither hormone by itself is, however, able to induce oestrogen secretion; for this the combined action of both hormones is necessary. This is rather puzzling, since ICSH gives pronounced cytological changes indicating increased activity in the oestrogen-producing cells. It is tempting to suggest that these cells cannot perform their secretory work until the granulosa has been activated by FSH. All other gonadotrophins are able by themselves to evoke oestrogen secretion, but all — with one exception (see below) — also have pronounced follicle stimulating activity. It thus seems possible that activation of both secretory elements in the follicle is necessary for hormone production. If so, it would add weight to the evidence for the view held in this paper on oestrogen secretion. In this connection it is of interest to note that — as has been pointed out by DAWSON and MCCABE (1951) and others — gonadotrophins are not able to evoke oestrogen secretion in the infantile rat ovary until a time (10—12 days of age) when antrum formation is commencing in the follicles.

HCG differs in many respects from the other gonadotrophins. It induces repair of the deficiency cells and oestrogen secretion in the ovary of the hypophysectomised mature rat without any obvious follicle stimulation. This does not fit, but does not therefore necessarily invalidate, the supposition of the interaction mechanism suggested above. HCG has been shown to possess a weak FSH activity (LYON *et al.* 1953) so that the contradiction may be only apparent. Another source of error is the existence of persisting corpora lutea in the ovary, which may be able to influence the oestrogen-producing cells. An experiment by GREEP (1938) suggests such a mechanism. He showed that when the persisting corpora were brought to involution by an ICSH preparation, HCG no longer induced any oestrogen secretion. Unfortunately, this interesting experiment has never been confirmed. In the hypophysectomised immature rat HCG brings about repair of the deficiency cells but — for unknown reasons — no oestrogen production. If, however, HCG is administered in combination with FSH, oestrogen is released. Thus

another example illustrating the general principle of an interaction as suggested above.

One of the most important contributions to the understanding of the theca interna — granulosa system and their role in oestrogen production has been made by WESTMAN (1929, 1934). His experiments seem to be the only ones previously performed to secure information of a more direct nature but — possibly on account of extremely difficult steps in the experimental procedure — the results have not been generally accepted. Since the experiments — made on rabbit ovaries — are too complicated to allow a brief description the reader is referred to the original papers or to the review of CORNER (1938). The experiments seem to yield evidence for the following conclusions: Theca interna produces oestrogen but not progestin, which is secreted by the granulosa. These two cell systems are not independent, however, but influence each other. Thus normal function of the progestin-producing cells appears to be dependent on the oestrogen-producing cells, probably by local action of oestrogen. Local action of the granulosa on the theca interna cells seems to be responsible for their proliferation as well as their oestrogen secretion. It would appear that the most important pertinent conclusion in WESTMAN's investigations is the following statement: "Solange die Thekazellen in Verbindung mit funktionierendem Granulosaepithel stehen, haben sie die Fähigkeit Follikulin zu produzieren." Thus, two entirely different experimental approaches — that of WESTMAN and that of the present study — to secure more direct evidence of the site of oestrogen formation in the ovary have led to the same general conclusion.

The experiments in the present study show that it is possible with the technique devised to dissect and transplant cell aggregates containing only one of the endocrine cell types in the ovary. This opens the possibility of studying the histogenesis and secretory function of ovarian tumours experimentally induced in grafts originally containing only one or two of these cell types.

SUMMARY

The present paper is concerned with an investigation on the site of production of oestrogen in the rat ovary by a method designed to yield more direct evidence than hitherto available.

1. A dissection technique was devised permitting isolation of small cell aggregates from the rat ovary containing only one endocrine cell system.

2. Such cell systems alone or in certain combinations were auto-transplanted to the anterior chamber of the eye of spayed animals, a contiguous vaginal autotransplant serving as an indicator of the oestrogenic activity of the ovarian grafts. The specificity of this indicator system to oestrogen is discussed.

3. As to the ovarian grafts, only in exceptional cases did they produce systemic hormonal effects, and interference by oestrogenic substances from other sources or by metabolites from steroids released in the grafts was excluded by the appearance of the vagina *in situ* and a control vaginal transplant in the other eye.

4. The following isolated cell systems were transplanted: interstitial cells, theca interna cells, corpus luteum cells and granulosa cells. All of these cell types were able to survive when grafted. In spite of the complex topography of the rat ovary, it proved possible to secure a certain number of grafts consisting of interstitial cells only or of theca interna cells only. Uncontaminated grafts of granulosa cells and corpus luteum cells could be readily obtained. The dissection technique thus provides a tool for studying ovarian tumours experimentally induced in grafts originally containing only one type of endocrine cells.

The transplanted granulosa cells were luteinised and formed cell aggregates of the same histological structure as the normal corpus luteum. The following combinations of cell systems were studied: interstitial cells + corpus luteum cells and theca interna cells + granulosa cells or corpus luteum cells.

5. Secretion of oestrogen was never recorded in transplants of the pure cell systems but only in transplants containing theca interna cells or interstitial cells *combined with* granulosa cells

or corpus luteum cells. The validity of these results is discussed and it is excluded that errors in the experimental method might have caused the negative results with the isolated cell systems.

Isolated follicles were not transplanted, but the material permitted an indirect analysis of the oestrogen-producing capacity of follicles in different developmental stages, showing that this probably arises with the development of the theca interna gland cells.

It was found that a single follicle with few theca interna cells could produce a local oestrogenic effect on the indicator epithelium, while a single follicle of preovulatory size and well-developed thecal gland was able to produce full oestrous reaction of the vagina and uterus *in situ*.

6. It is concluded from these results that the production of oestrogen is dependant on an interplay between theca interna gland cells or interstitial cells and corpus luteum cells or granulosa cells.

7. The primary source of oestrogen in the ovary is discussed against the background of the literature and the present findings. It is concluded that the theca interna gland cells and the interstitial gland cells most probably constitute the oestrogen-producing system in the ovary but that these cells are functionally dependent on the system granulosa-corpus luteum cells.

8. The possibility of intra-ovarian mechanisms operating in both directions between the two principal cell-systems — the oestrogen- and the progestin-producing — is discussed.

Acknowledgements

I wish to express my gratitude to Professor Gösta Glimstedt and Associate-Professor Nils-Åke Hillarp for guidance and constructive criticism throughout the investigation.

Acknowledgement is due to Miss Inger Falck, Miss Britt Jönsson and Mr Georg Thieme for valuable technical assistance.

The investigation was supported by grants from *Riksföreningen mot Cancer* and from the Faculty of Medicine, University of Lund.

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